

Studies on Erythropoiesis
and Total Red Cell Volume in
Congestive Heart Failure

BY

SVEN HEDLUND

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PREFACE

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Stockholm, March 1953.

Sven Hedlund

ABBREVIATIONS

TRCV	=	Total red cell volume
EB	=	Evans blue dye
MCD	=	Mean corpuscular diameter
Hct	=	Hematocrit
Hb	=	Hemoglobin
RBC	=	Red blood corpuscle count
MCH	=	Mean corpuscular hemoglobin
MCHC	=	Mean corpuscular hemoglobin concentration
MCV	=	Mean corpuscular volume
M.	=	Male
F.	=	Female

INTRODUCTION

In the pathophysiology of cardiac insufficiency, problems connected with the blood volume occupy a dominant position. Many papers have been published on this subject, in which for the most part, the investigations have been performed by dye methods. Considering that the blood volume cannot be regarded as an entity, a distinction should be made between cell volume and plasma volume and different methods must be applied for their respective determinations. Since, in the present investigations, attention has chiefly been focussed on variations in the total red cell volume in cardiac insufficiency, the P^{32} -method has appeared to be suitable.

In order to investigate the complex problem of the formation of blood corpuscles and the changes in the total red cell volume in cardiac insufficiency, an extensive clinical survey was planned along the following lines:

- 1) The erythropoiesis including some investigations into its regulation.
- 2) The total red cell volume, the erythrocytes themselves (*MCD*, *MCV*, *MCHC*, *MCH*) and the number of reticulocytes.
- 3) The destruction of the red blood corpuscles, based on studies of changes in the osmotic resistance of the erythrocytes and of changes in serum iron.

Emphasis has, accordingly, been placed on the erythropoiesis and its resultant total cell volume and size of individual blood corpuscles. A separate problem is the hemoglobin synthesis which, in the present work, has only been studied in brief.

In order to investigate the formation of the blood corpuscles, the bone marrow has been examined in decompensated cases. A differentiation of the erythroid cell elements has been carried out, and the number of mitoses and cellular diameters have been studied. The influence of anoxemia has been observed by means of oxygen analyses of arterial blood and the information thus obtained led to certain animal experiments (published by Plum and Hedlund in 1952).

In the peripheral blood, the course of the reticulocyte curve has been followed during the treatment of patients suffering from cardiac insufficiency and its correlation to the arterial oxygen saturation studied.

An account is also given of the total red cell volume in decompensation and compensation. Particular attention has been paid to certain factors that may, conceivably, affect that volume in decompensation, such as the type of the cardiac affection, the age and sex of the patient, the duration of the symptoms of a manifest insufficiency and, finally, the actual degree of decompensation, as far as it can be ascertained from heart volume, venous pressure, edema and other sequelae of stasis. The effect of anoxemia has likewise been observed.

The same factors and others, e.g. the condition of the liver, and their influence on the mean diameter, have also been studied as far as the individual erythrocytes are concerned. The information gained from this clinical material has been supplemented with the results of the forementioned animal experiments (Plum and Hedlund, 1952).

The destruction of erythrocytes, as manifested in changes in the osmotic resistance of the blood corpuscles and in the serum iron, has also been studied during the treatment from decompensation to compensation.

In addition, a comparison has been made of the mixing conditions in the circulation of P^{32} -labelled blood corpuscles and Evans blue, as well as of the total cell volume as determined by these two methods, respectively.

The investigation may be complicated by the various difficulties encountered in a material dealing with cardiac insufficiency. Such a material must be representative of different degrees of insufficiency. The diagnosis must be accurate, and the degree of severity of the decompensation uniformly assessed. The therapy must be adequate and rationally pursued, in order to give all cases an equal therapeutic chance. Unforeseen complications may adversely affect the course of treatment with the result that all the desirable information may be lacking.

The comprehensiveness of the research is justified by the fact that, previously, only a number of limited investigations have been performed — independent of one another and devoted only to part of these complex problems. The survey of the literature has been divided into two main groups, corresponding to those of this work but owing to the magnitude of the subject cannot pretend to any completeness.

It has not been possible in the present work to enter into all problems, some have only been studied in brief, a continued and extended study being required.

Medico-statistical analyses have been employed to a considerable extent in the interpretation of the numerical results of the investigations — but throughout, the statistical analyses have only been interpreted in the light of the medical relevancies. Every numerically noted tendency has been subjected to different statistical tests. In the interpretation of the test results, regard has, as a matter of course, been paid to the possible effect of deficiencies in the representativeness and size of the material. Still, it should be borne in mind that, since only low absolute frequencies have been observable, differences and correlations cannot invariably be supposed to be “statistically proved”. The data derived from the statistical analyses can, however, be correctly evaluated only when placed in their respective medical relations.

The case reports will be available in the library of Karolinska Institutet, Stockholm.

CHAPTER I

Cell and plasma volume in circulation

Cell, plasma and blood volume studies form an extensive literature.

Exhaustive accounts of the various methods for determining the blood, plasma and cell volume have been presented earlier by PLESCH (1909), ERLANGER (1921), SEYDERHELM & LAMPE (1925), FLEISCHER-HANSEN (1928), ROWNTREE & BROWN (1929) and REEVE (1948).

The earliest, so-called direct methods involved the collection of blood from dead persons. A special method was worked out by WELCHER (1854) and further improved by PLESCH (1909) and others.

The indirect methods are applied to living persons. The basic principle is to introduce a test substance into the blood course whose dilution there will allow a determination of the amount of blood. Attempts have been made to find indicators that are not eliminated before mixing in the blood stream and whose concentration is easily established. Thus, by using substances that are distributed in the plasma, a proper determination of the plasma volume is rendered possible (i.e. methods for determination of plasma volume). Further, by introducing specially labelled erythrocytes into the blood stream, the total red cell volume can be estimated (i.e. methods for determination of red cell volume). The blood and plasma volume are obtained secondarily from the determined red cell volume by means of the venous hematocrit.

METHODS FOR DETERMINATION OF PLASMA VOLUME

The injection of different dyes in the circulation has above all been used. The dye method was introduced by KEITH, ROWNTREE and GERAGHTY in 1915. They used vital red. HOOPER, SMITH, BELT & WHIPPLE (1920) worked out a method for brilliant vital red. HARRIS (1920), GRIESBACH (1921) and others adopted Congo red, and SEYDERHELM & LAMPE (1925) trypan red and trypan blue.

DAWSON, EVANS & WHIPPLE (1920), in a comprehensive investigation of 60 different dyes, found that the blue azo dye (T-1824) was the best dye available for these tracings.

Evans blue-method.

Behaviour of the dye.

Evans blue or T-1824 which is easily soluble in water has been shown to be combined with the albumins (EFSKIND, 1940; RAWSON, 1943), and at particularly high temperatures also with the globulins.

LEVEEN and FISCHMAN (1947) inferred that T-1824 forms a dissociable compound with the plasma albumin and globulin. In their opinion, unionized dyes pass to the capillary membranes, to be fixed to tissue protein. This may explain the rapid early phase of disappearance. Elimination of Evans blue through the capillary wall takes place either in combination with albumin (RAWSON 1943) or in a free state (LEVEEN and FISCHMAN 1947).

BARNES, LOUITT and REEVE (1948), however, considered that as the rate at which Evans blue combines with albumin was considerable much was not likely to be lost.

A relatively slow rate of disappearance of isotopically tagged plasma protein has been recorded by FINE and SELIGMAN (1944), HEIDELBERGER and others (1942), FINK and others (1944) and CRISPELL, PORTER and NIESET (1950) in contrast with the more rapid disappearance of dyes.

Many investigations deal with the diffusion into the lymph and the reticulo-endothelial system. T-1824 appears in the lymph about 20 minutes after the injection, increasing in amount with time (CARDOZO, 1940; FERREBEE, LEIGH and BERLINER, 1941; COURTICE, 1943). Not more than 2 per cent will return to the blood within 1 hour (FERREBEE LEIGH and BERLINER). GIBSON & EVANS (1937) and PERERA failed to find any trace of Evans blue in edematous fluid, pleural transudates and ascites in cases of cardiac insufficiency, and cirrhosis of liver. In cases of capillary injuries, a higher rate of disappearance of the Evans blue has been observed (FREEMAN and WALLACE, 1938; FERREBEE, LEIGH and BERLINER, 1941; COPE and MOORE, 1944). MAURER has shown that, in anoxia, the dye excretion increases considerably through the lymph.

Evans blue is not bound to the red blood cells (GREGERSEN and SHIRO 1938, SHOHL and HUNTER, 1941, COURTICE, 1943), nor to the white blood corpuscles (SHOHL and HUNTER, 1941).

Mixing and disappearance of Evans blue in the circulation.

Concentration curves regarding Evans blue illustrate two processes, viz, mixing and disappearance of the dye in the circulation. Since these two processes can take place simultaneously, the curves may afford certain difficulties in interpretation. Many theories have been propounded. Generally GIBSON's and EVANS' (1937) explanation has been accepted. They maintained that the curve consists of two main parts, a mixing curve representing the steeper fall during the first few minutes, and a disappearance slope indicative of a slower loss of dye from the circulation due to factors already mentioned here. The point of intersection of these two curve parts, i.e. mixing time, denotes the time for mixing in the circulation.

This schema for explanation has been subject for much discussion and criticism. Thus KENNEDY & MILLIKAN and CRUICKSHANK & WHITFIELD (1945) maintained that T-1824 leaves the circulation immediately after the injection, i.e. already during the mixing phase, and is absorbed by some system. ROBINOW & HAMILTON (1940) were of the opinion that mixing was already established after 1 minute. The remaining part of the curve

they interpreted as due to a loss of dye in the lymph. However, this is considered by a number of investigators as unlikely (COURTICE; CARDOZO; FERREBEE, LEIGH & BERLINER) on the basis of analyses of the dye concentration in the lymph.

Using another method, namely injection of undyed plasma into dogs, dyed with T-1824, LAWSON, OVERBERG, MOORE & SHADLE (1947) found that mixing was complete within 2–5 minutes. The same length of time was required for mixing of injected labelled cells. Nor did they notice any initial rapid fall of Evans blue, in contrast to the results obtained by the methods commonly used.

The percentage amount of dye leaving the circulation per hour has been calculated by several researchers from the disappearance slope. DAWSON, EVANS & WHIPPLE (1920) determined the disappearance rate as 5–8% per hour, GIBSON & EVANS (1937) as 6%, NOBLE & GREGERSEN as 5.2% and GREGERSEN & LAWSON as 8.8% loss.

For interpretation of the obtained curves of Evans blue, extrapolation methods have been used, thus linear extrapolation (GIBSON & EVANS, 1937; FERREBEE, LEIGH & BERLINER, 1941; REEVE & ARNIM, 1946; CRUICKSHANK & WITHFIELD, 1945, and others), semi-logarithmic extrapolation (GREGERSEN & RAWSON, 1943; PRINCE & LONGMIRE, 1942; NOBLE & GREGERSEN, 1946, and others) double logarithmic extrapolation to 1 minute (OVERBERG, MOORE, SHADLE & LAWSON, 1947; McLAIN, RUHE & KRUSE, 1951) and last the root extrapolation method (KING, COLE & OPPENHEIMER, 1942). The accuracy of the methods has been a subject for discussion, and comparisons of the methods have been performed.

GREGERSEN & RAWSON (1943) and v. PORAT (1951), are of the opinion that the linear and semilogarithmic extrapolations give practically identical values of the plasma volume.

Some investigators have, instead of using the extrapolation method for calculation of the plasma volume, based their determinations on the concentration of dye in single blood samples. Difference in the plasma volume has been noted when calculated from the latter method as against linear extrapolation. (GILDER, MÜLLER & PHILLIPS, 1940; GREGERSEN, 1944; NOBLE & GREGERSEN, 1946; MCLEMAN & THORIM, 1948; v. PORAT, 1951).

Determination of Evans blue in the blood samples.

Evans blue can either be determined directly in plasma or serum by colorimeter or spectrophotometer, or indirectly through an extraction process by means of which the dye is absorbed by suitable solvents. The first-mentioned method is the one generally adopted.

Detailed investigations regarding the conditions of absorption of Evans blue, dissolved in water, saline or plasma, have been performed. The binding to protein causes plasma solutions to exert a stabilizing effect on the absorption curve of Evans blue as regards changes in the *pH* of the solution and deviations in the saline and protein concentration (GREGERSEN & GIBSON, 1937, EFSKIND, 1940, HEILMEYER, 1929).

Standard solutions should, therefore, be prepared on plasma or serum solutions of Evans blue. It is sufficient to make a standard curve of different concentrations of Evans blue for each preparation of dye (GREGERSEN & GIBSON, among others).

Disturbing influence from lipemia, hemolysis and residual dye in the samples is compensated by a spectrophotometer where the readings for Evans blue take place at 620 m μ , while oxyhemoglobin has its maximum at 540 m μ (GREGERSEN, GIBSON & STEAD, 1935, GIBSON & EVANS, 1937, HEILMEYER, 1929, EFSKIND, 1940). Lesser degrees of hemolysis do not exert any disturbing effect on the readings at 620 m μ as the absorption for oxyhemoglobin is very slight at this wave-length. To eliminate the effect of a stronger hemolysis that disturbs absorption of Evans blue at 620 m μ , theoretical corrections have been elaborated (GIBSON and EVANS, 1937). The value of these corrections has been called in question (PHILLIPS, 1943, NOBLE & GREGERSEN, 1946). It seems more justifiable to exclude entirely samples with marked hemolysis.

METHODS FOR DETERMINATION OF TOTAL RED CELL VOLUME

Agglutination methods.

TODD and WHITE (1912) introduced a method for determining the amount of blood corpuscles by an agglutination process.

A similar, improved method was advanced by W. ASHBY (1919). The technique, which calls for much experience, has been further improved and modified by, DACIE and MOLLISON (1943), OSBORNE & DENSTEDT (1947), and BARNES, LOUTIT & REEVE (1948), among others.

HUCH & BIGALOW (1923), VISCHER (1928), KIRKEGAARD & LARSEN (1943) and HEDENSTEDT (1947) studied elliptocytes after transfusion to normal subjects. HEDENSTEDT (1947), on the basis of these studies, presented a method for determining the total red cell volume.

The CO-method.

The amount of hemoglobin in the body has been estimated by letting a definite amount of CO combine with the hemoglobin, and thereafter determining the COHb-concentration. The original method, introduced by GREHAUT and QUINCAUD (1882), has been improved and modified several times, both as far as the inhalation apparatus, and the determination of COHb are concerned. (HALDANE & SMITH, 1899; VAN SLYKE & SALVESEN; HEILMEYER; HARTMAN, 1937; STEINMANN, 1938; WENNESLAND, 1940; ROUGHTON, 1941; MAXFIELD, BAZELT & CHAMBERS, 1941; SCHOLANDER & ROUGHTON, 1943; ROOT, ROUGHTON & GREGERSEN, 1946; SJÖSTRAND, 1948; COURTICE & GUNTON, 1949, and others).

A lot of work dealing with special problems connected with this method has been performed, i.e. the inhalation period, the mixing conditions, the necessity to calculate COHb before the experiment, the absorption of CO by myoglobin. During recent years the most comprehensive studies have been performed by ROOT, ROUGHTON &

GREGERSEN (1946) and SJÖSTRAND (1948). With the new method, introduced by SJÖSTRAND (1948), a number of clinical investigations have been carried out. (SJÖSTRAND and SJÖSTRAND and co-workers).

According to ROOT, ROUGHTON & GREGERSEN and SJÖSTRAND about 2–3% of CO combines with myoglobin. CO also combines with hemoglobin of the bone marrow (GREGERSEN & ROOT, 1950). In the opinion of GREGERSEN & ROOT (1950), the difference in volume of cells tagged with radioactive iron and CO (NICKERSON, SHARPE, ROOT, FLEMING & GREGERSEN, 1950) is of the order of magnitude that would be expected from estimates of the amount of red marrow.

Cell-tagging methods by isotopes

Radioactive isotopes of iron, phosphorus, potassium, chromium and Thorium B were used for labelling the red blood corpuscles. The labelled blood corpuscles are injected into the blood stream and, after mixing, the radioactivity of the erythrocytes is determined in the drawn blood samples. Total red cell volume of the subject is then ascertained from the values obtained, viz, the activity and volume of injected corpuscles and the corpuscular activity of drawn samples.

Radioactive iron.

After having established that radioactive iron is incorporated in hemoglobin, and that its concentration in the erythrocytes remains constant for a long period (HAHN, BALE, LAWRENCE & WHIPPLE, 1938, 1939; HAHN, BALE, ROSS, HETFIG & WHIPPLE, 1940; HAHN, ROSS, BALE & WHIPPLE, 1940; HAHN, BALE & BALFOUR 1941/42, and CRUZ, HAHN & BALE 1941/42), the prerequisites for an erythrocyte determination method are in evidence. Thus, HAHN, ROSS, BALE, BALFOUR & WHIPPLE (1941) introduced a method where blood containing erythrocytes, labelled with radioactive iron from a donor dog, was injected in the test animal whose total red cell volume was to be determined. The technique has been further refined by PEACOCK, EVANS, IRVINE, GOOD, KIP, WEISS & GIBSON (1946). HAHN et al. (1941) concluded that mixing had taken place after 2 minutes. GIBSON, PEACOCK, SELIGMAN & SACK (1946) and MENEELY, WELLS & HAHN (1947) applied the method to human beings.

By means of this method, a test substance has been obtained that is constant in the blood stream for a very considerable time, thus allowing to carry out red cell volume determinations even after a lapse of several days after the injection of labelled corpuscles. On the other hand, this method requires a group of donors who have been charged with radioactive iron for an appreciable time. Furthermore, subjects with different blood groups require different donors. These donors are exposed to radiation emitted by Fe^{59} for a very appreciable time, as the radio-iron is almost quantitatively conserved in the organism and disappears only through radioactive decay, which takes place with the half-life time of 47 days.

Radioactive phosphorus.

Distribution of radiophosphorus.

After the injection of the radiophosphorus, the deposit of P^{32} in different organs has been studied in animals (HEVESY et al.; GAUNT, GRIFFITH & IRVING, 1941; JONES, CHAIKOFF & LAWRENCE, 1940; COHN & GREENBERG, 1938, and others) and in patients at autopsy (LAWRENCE, SCOTT & TUTTLE, 1939 and ERF, 1941).

From these investigations it was found that the uptake of radiophosphorus is rather rapid, with varying distribution in different organs for varying periods of time. Bone and muscles show the greatest uptake. Within the first 6 days after the administration of radiophosphorus, normal persons discharged 25–50 per cent of the injected activity, mainly via the kidneys (ERF, LAWRENCE, 1941; HEVESY, HAHN & REBBE, 1939).

Uptake of radiophosphorus in the erythrocytes.

As stated by HEVESY & co-workers, as well as by HALPERN; EISENMAN, OTT, SMITH & WINKLER, the activation of the blood corpuscles is due to an exchange between the inorganic phosphate of the plasma and that of the blood corpuscles, and by an incorporation of inorganic corpuscle phosphate into organic acid-soluble phosphorous compounds. The above-mentioned authors have shown that the rate of passage of phosphate into the corpuscles is low, while the phosphate ions which penetrate the corpuscles rapidly participate in phosphorylation processes. The transfer may take place from one organic phosphate group to another, without passing through an inorganic stage (HEVESY et al., 1938; PARNAS, 1939). Different factors influencing these turnover processes are demonstrated (HEVESY & co-workers; TULIN, DANOWSKI, HELD & PETERS and others). Thus it has been shown, that the erythrocytes are but insignificantly permeable by phosphate at $3^{\circ} C$ and the permeability is considerably accelerated at $37.5^{\circ} C$ (about 20–30 times) (HALPERN, 1936; EISENMAN et al., 1940; SOLOMON, HALD & PETERS, 1940; HAHN & HEVESY, 1942). A scheme of the phosphate metabolism in the blood corpuscles is presented by Guest & Rapoport (1939).

The intrusion of P^{32} into the corpuscles is an interchange process, the entrance of a phosphate ion being compensated by the emigration of another. In an earlier phase of the experiments the corpuscles hardly contain any radioactive phosphate. The entrance of a radioactive phosphate ion is therefore bound to be compensated by an extrusion of a non-radioactive one. This process must clearly lead to an accumulation of radioactive phosphate added to the plasma in the blood corpuscles. If the ratio between radioactive and non-radioactive phosphate in the plasma and in the blood corpuscles becomes equal, clearly no further concentration of P^{32} in the corpuscles will take place.

In vitro experiments the specific activity of corpuscles is always much lower than the specific activity of the plasma phosphate. The fact that phosphate penetrates fairly slowly into the blood corpuscles but gets rapidly incorporated into labile acid soluble compounds (HEVESY & ATEN) has the effect that the pool available to the intruded phosphate thus gets very appreciably enlarged. The intruded radioactive phosphate is not only distributed among the inorganic phosphate ions present in the corpuscles

but also among the labile acid-soluble organic molecules present. The result of this distribution is that the rate of extrusion of radioactive phosphorus into inactive plasma — in blood volume determinations the radioactive corpuscles are injected into inactive human circulation — is very much slower than it would be without such a distribution.

As the concentration of easily renewable organic acid-soluble phosphorus compounds in the corpuscles is about 10–15 times that of the inorganic phosphorus of the corpuscles, the pool available in the corpuscles for the intruding inorganic phosphate is about 10–15 times larger than it would be if the intruded phosphate would be distributed only among the inorganic ions present.

The relation between the quotient of intrusion/extrusion of phosphate into and from the red corpuscles is discussed in detail by H. BOHR. He has also shown that the maximum in the activation of the corpuscles *in vitro* was reached after the lapse of 5 hours. If the time of intrusion is extended the activity of corpuscles successively diminishes. The decrease can be prevented by adding glucose to the blood sample (H. BOHR, 1950).

Shortly after the administration of labelled phosphate, tagged phosphatides penetrate from the liver into the circulation. Only 1 per cent of the blood corpuscle phosphatides were exchanged for plasma phosphatides after $3\frac{1}{2}$ hours (HAHN & HEVESY, 1939).

Five hours after the injection of plasma containing labelled phosphatides into the circulation of the dog, ZILVERSMIT, ENTENMAN, FISHLER & CHAIKOFF (1943) found 1.15 to 1.5 per cent of the labelled phosphatides in the red corpuscles.

Certain investigations in lactating cows (SMITH, 1938) indicate that an appreciable release of phosphatides from the corpuscles to the plasma does not occur.

Application of erythrocytes containing radiophosphorus for determination of red cell volume.

On the basis of reviewed studies concerning the uptake of radio phosphorus in blood corpuscles, HAHN & HEVESY (1940) presented a method for determination of the amount of blood corpuscles. Thus, first a donor was labelled with P^{32} (experiment on rats), and labelled blood was then injected into another rat. (The labelled blood cells of the donor contain labelled phosphatides as well as acid-soluble phosphate esters). The determination of the erythrocyte quantity was either based on an analysis of the phosphatides or of the acid-soluble phosphate esters.

BROWN, HEMPELMANN & ELMAN (1942), in a similar way, incubated dogs as donors. Labelled blood corpuscles from them were injected into the animal whose blood volume was to be determined.

GOVAERTS (1942) determined the blood volume in two males by the injection of labelled blood corpuscles and plasma from a donor.

HEVESY & ZERAHN (1942) simplified the method by measuring the activity of the inorganic phosphate in injected and withdrawn blood corpuscles, while simultaneously a new technique was developed for the labelling of the erythrocytes of the subject *in vitro*. The whole blood thus labelled is injected into the subject whose total red cell volume is to be established. They found a slight decrease in the activity of the withdrawn blood corpuscles 15 minutes after the injection into rabbits.

The method presented by HEVESY & ZERAHN (1942) has been applied to Man by several authors (NYLIN & MALM, 1943, 1944; HEVESY, KÖSTER, SØRENSEN, WARBURG & ZERAHN, 1944; NYLIN, 1945, 1946, 1947; NYLIN & BIÖRCK, 1947; NYLIN & PANNIER, 1947; NYLIN & HEDLUND, 1947, 1948, 1949, 1950; NYLIN & LEVANDER, 1948; KELLY, SIMONSEN & ELMAN, 1948; NYLIN & CELANDER, 1950; SUTTON, KARNELL & NYLIN, 1950; NYLIN & WADE, 1951) to a number of methodical and circulatory problems.

There are some methodical differences. Thus, NYLIN (1945) modified the technique for preparation of withdrawn blood samples which are shown to provide the same corpuscular activity whether they are dried or ashed. KELLY, SIMONSEN & ELMAN hemolysed the blood corpuscles and left them to dry on object slides.

By using this method it has been found that the corpuscular activity in the circulation is rather constant for up to one hour after the injection (NYLIN; NYLIN & HEDLUND; KELLY, SIMONSEN & ELMAN). HEVESY, KÖSTER, SØRENSEN, WARBURG & ZERAHN, however, found that the blood corpuscles begin to lose their activity 15 minutes after the injection.

From the distribution curve of corpuscular activity by rapid arterial blood sampling, NYLIN has assumed that mixing in normal cases seems to take place as soon as after a couple of minutes. In this respect HEVESY, KÖSTER, SØRENSEN, WARBURG & ZERAHN agree.

The original method by HEVESY & ZERAHN has been modified in other respects, too. Thus, the activated corpuscles are washed twice or three times, resuspended in saline or inactive plasma, and after that the mixture is injected (ANDERSSON, 1942; NYLIN, 1945; NIESET, PORTER, TRAUTMAN, BELL, PARSON & MAYERSON, 1948; REEVE & VEALL, 1949; NYLIN & HEDLUND, 1949; NACHMAN, JAMES, MOORE & EVANS, 1950; BERLIN, LAWRENCE & GARTLAND, 1950; REID & ORR, 1950; MOLLISON, VEALL & CUTBUSH, 1950; WASSERMAN, TSE-FEI YOH & RASHKOFF, 1951; MUNKHERJEE & ROWLANDS, 1951; NYLIN & WADE, 1951, and others). There are some differences in the measurement technique of the activity of the blood samples used by the investigators.

The loss of the corpuscular activity when injecting washed corpuscles has been calculated to be 5% within 30 minutes (NIESET et al.), and within one hour 4% (WASSERMAN et al.), or 6–7% (REEVE & VEALL), or 8.5% (NYLIN & WADE) and within 70 minutes 10% (MUELLER & HASTINGS). The activity has been found, however, to be strikingly constant within one hour by NACHMAN et al. in man and MUNKHERJEE and ROWLANDS in dogs. ANDERSSON, who made his experiments in rabbits, noted a loss of activity of 8% after 20 minutes.

Most investigators emphasize that the inherent error of the method is within 5% in the cell volume.

Other isotopes.

In recent years other isotopes have been used for the labelling of red corpuscles. Thus, HEVESY & NYLIN (1951) as well as YALOW & BERSON (1951) have applied K^{42} labelled corpuscles in erythrocyte volume determinations. After labelling the erythrocytes in

vitro at 37° for 2 hrs, the blood sample was injected into the patient. In the course of 1 hr, within the error of the measurements, no change in the activity of the red corpuscles could be observed. During the same time washed labelled red corpuscles injected into the circulation lost on an average 3.5% of their K^{42} content (HEVESY & NYLIN).

By leading thorium emanation through blood for a few minutes red corpuscles labelled by the presence of the active deposit of thorium are obtained (HEVESY 1951). Most of the Thorium B formed in the plasma is taken up by the red corpuscles. Within the first 2 hrs after the injection of Th B labelled blood into the circulation loss of Th B by the labelled corpuscles is almost negligible. The method is applied to erythrocyte volume determinations by HEVESY & NYLIN (1951).

In 1950 a method for determination of the total red cell volume in man by radioactive chromium was introduced by STERLING & GRAY. Tagged erythrocytes, incubated in vitro for 1 hr, and injected into the circulation, lose about 5 per cent of their radioactivity to the plasma within 24 hrs.

COMPARISON BETWEEN ISOTOPE AND DYE METHODS

In *table 1* are summed up the results of simultaneous determinations of the cell volume by the isotope and agglutination methods and the dye — venous hematocrit — method.

When using the same correction factor for trapped plasma, the erythrocyte volume, estimated with dye, is about 13% higher than with any of the isotope-methods or agglutination methods. BERLIN et al. have further shown in experiments in mice that there is no significant difference in the determination of the blood volume when using radio phosphorus or radio iron. Blood volumes estimated with the aid of P^{32} also give about the same values as with the aid of K^{42} (YALOW & BERSON). When using P^{32} and Thorium B labelling technique HEVESY & NYLIN (1935) obtained corresponding values in the total red cell volume. The discrepancies between the tagging methods and the dye-hematocrit method may be explained from systematic errors such as

- 1) overestimation of the plasma volume with Evans blue,
- 2) underestimation of red cell volume with tagging methods (Fe^{59} , P^{32} , K^{42} and agglutination).
- 3) different distributions of red corpuscles and plasma in the blood stream in the body.

In an earlier review of the Evans' blue method, it has been pointed out that an overestimation of the plasma volume may occur when using unsuitable calculation- and determination methods. Still, the reviewed discrepancies in the erythrocyte volume do not seem explainable simply from this fact.

There are no convincing investigations proving that the tagging methods underestimate the cell volume. An underestimation of the red cell volume cannot be due to a loss of the radioactive label from the corpuscles as such a loss would result in an increased figure for the red cell volume.

Table 1. Comparisons between cell tagging methods and Evans blue in estimating total red cell volume in humans, as well as between body and venous hematocrit.

	Number of cases	Ratio TRCV Tg TRCV EB	Ratio BodyHct Ven.Hct.	Correction for trapped plasma	Material
<i>Radioactive iron and Evans blue:</i> Gibson J. G., Peacock W. C., Seligman A. M. & Sack T. (1946)	40 m.	0.85	0.91	no corr.	normal subjects
Meneely G. R., Wells E. B. & Hahn P. F. (1947)	28 m.	0.81	0.89	no corr.	normal & convalescents
<i>Radioactive phosphorus and Evans blue:</i> Hevesy G., Köster K. H., Sørensen G., Warburg E. & Zerah K. (1944)	8 cases	0.86		no corr.	normal subjects
Mayerson H. S., Lyons C., Parsons W., Nieset R. T. & Trautman W. (1948)	26 m. & f.	0.96	0.98	f = 0.915	normal & convalescents
Reeve E. B. & Veall N. (1949)	13 cases	0.87		f = 0.95	normal
Nachman H. M., Watson James G., Moore J. W. & Evans E. J. (1950)	38 m. & f.	0.80 0.87	0.88	no corr. f = 0.96	normal & convalescents
Mollison P. L., Veall N. & Cutbush M. (1950)	35 norm. infants		0.87	f = 0.95	
Wasserman L. R., Yoh Tse-fei, & Rashkoff I. A. (1951)	30 m. & f.		0.85 0.92	no corr. f = 0.915	normal & convalescents
<i>Agglutination and Evans blue:</i> Barnes D. W. H., Loutit J. F. & Reeve E. B. (1948)	20 cases	0.88		f = 0.95	normal & patients
Mollison P. L., Veall N. & Cutbush M. (1950)	3 infants		0.88	f = 0.95	

Abbreviations: TRCV Tg = total red cell volume acc. tagging methods;
 TRCV EB = total red cell volume acc. Evans blue method;
 m = males; f = females; Hct = hematocrit.

Assuming that tagging methods lead to a correct value of the cell volume and that Evans blue method gives the correct value of the plasma volume, we arrive at a correct blood volume figure by addition of these two data.

The blood volume has also been calculated from the plasma volume and the venous hematocrit. Values thus obtained were found to be larger than those obtained by addition of plasma and cell volume. This discrepancy leads to the conclusion that body hematocrit is not identical with the peripheral hematocrit. Body hematocrit is defined as follows:

$$\frac{\text{cell volume (tagging method)}}{\text{cell volume} + \text{plasma volume (Evans blue)}}.$$

When computing the blood volume from cell and plasma volumes the venous hematocrit is not to be considered. This must, however, be done when calculating the blood volume from the plasma volume. The ratio body hematocrit/venous hematocrit is on an average 0.9. The explanation is discussed in the following.

Distribution of erythrocytes and plasma in the circulation.

SMITH, ARNOLD & WHIPPLE (1921) arrived at an early date at the conclusion that the amount of blood corpuscles is relatively lower in the small vessels than in the large ones and that, for this reason, the jugular vein yields a higher hematocrit than the average hematocrit obtained from cell and plasma volumes, as determined simultaneously. The authors suggest that the blood corpuscles move with the axial stream, and that 44% of the transverse area consists of rather slowly flowing films of plasma. They estimate this so-called stagnating plasma at 9 per cent of the total blood volume.

By making blood pass through glass tubes of successively decreasing calibre, FÄHRAEUS (1926) studied the distribution of blood corpuscles and plasma, respectively. Later his observations have been applied to the circulation conditions in man (FÄHRAEUS; FÄHRAEUS, LINDKVIST; WEJLENS). They have found that in the minute blood vessels, the erythrocytes follow the more rapid axial course, while the plasma moves with the slower marginal stream. Fåhræus regards these narrow vessels of a diameter of between 0.020 and 0.300 mm. termed paracapillaries as a functional unity. Through hydrodynamic forces, the blood corpuscle is driven by the plasma stream from the vascular wall towards the centre, at a distance determined by the size of the vessel. The erythrocytes, accordingly, circulate much more rapidly than the plasma, a fraction of which appears to remain practically motionless in certain vascular systems.

In the opinion of HAHN, ROSS, BALE, BALFOUR & WHIPPLE (1942) injected dyes will only very gradually be transferred from rapidly circulating plasma in the axial stream to the slow-flowing, and partly stagnated, marginal film of plasma. The plasma of this layer is estimated by the authors at 21 per cent of the total volume of plasma, though it must in no way be regarded as a separate unity, since it remains in a constant, though slow, intercourse with the more swiftly circulating plasma.

EBERT and STEAD (1941) showed that a hemoglobin concentration of blood milked from the minute vessels in males was from 0.8 to 1.8 gm, i.e. 8 per cent, lower than the hemoglobin concentrations of venous blood. Thus, these blood vessels contain more plasma.

By means of radioactive isotopes of iron and iodine, GIBSON, SELIGMAN, PEACOCK, AUB, FINE & EVANS (1946) found that 17 per cent of the total blood volume was to be found in the small vessels, arteriole, capillaries and venules, a somewhat greater proportion of the total plasma volume and a somewhat smaller proportion of the total of red cells being contained in the minute vessels. In the small vessels, 7 per cent of the total of blood occurred in the skeletal musculature, 5 per cent in the liver, 1–2 per cent in the spleen, lungs, kidneys and gastro-intestinal tract, and less than 1 per cent in heart and brain. They also noted that, at an average arterial hematocrit of 43, the hematocrits are, approximately, in the spleen 80, in the liver 40, in the lungs 35, in heart and skeletal musculature 20–25, and in kidneys, gastro-intestinal tract and brain 15–20. The ratios of the mean body hematocrit and the hematocrits of blood from small and from large vessels to the arterial hematocrit, respectively, keep at 0.85, 0.70 and 0.9.

Using the forementioned technique, the same authors have also investigated the ratio between rapidly circulating erythrocytes and the total amount of erythrocytes in the vascular system of different organs, arriving at an average of 1.01 ± 0.05 . From this, they conclude that in normal dogs all blood corpuscles in the vascular system are in active circulation. EBERT & STEAD (1941) come to the same conclusion in their investigations, as well as HAHN, ROSS, BALE, BALFOUR & WHIPPLE (1942).

Summing up, the results of the above investigations show that the distribution of blood corpuscles and plasma, respectively, varies from one vascular section to another, and that the circulatory conditions of blood corpuscles and plasma differ from one another.

Accordingly, a determination of one of these components cannot bear out any definite conclusions regarding the other.

In order to ascertain the total red cell volume, methods directly serving this purpose should be applied, while plasma methods should be adopted for calculations of the amount of plasma, i.e. methods appropriate to the particular problem to be solved should be employed.

Blood depots.

The question of the so-called blood depots is intimately connected with investigations concerning the calibre variations in the vascular system.

Certain organic and venous systems belonging to the peripheral circulation were earlier considered to be blood depots and, apparently, verified by heating, exercise tests and adrenalin injections which caused an increase of the plasma volume in animals as well as in human subjects. The depot organs were specified as the spleen (BARCROFT, 1921, BARCROFT et al., ABDERHALDEN & LAUDA, HAAM, VOLICER & VESIN, BINET, and others), the liver (GRAB, JANSEN & REIN 1929, MATTSSON 1929, DALE & BAUER 1932, and others), the subpapillary capillaries in the skin (WOLLHEIM 1927), the venous system,

particularly in the splanchnic area (GOLLWITZER-MAYER 1930, FLEISCH & others), or in the muscles GABBE & OVERHOF 1936).

The occurrence of closed depots of blood in Man in Barcroft's original sense — at least of a capacity worth mentioning — has been called in doubt by many authors using exercise tests and adrenalin injections with isotope tagging, or other methods (LAMSON, 1920, CHANG & HARROP 1927, EBERT & STEAD 1941, ASMUSSEN 1941, KALTREIDER & MENEELY 1940, HAHN, BALE & BONNER 1942, GIBSON, SELIGMAN, PEACOCK, AUB, FINE and EVANS 1946, NYLIN 1946, 1947, PARSON, MAYERSON, LYONS, PORTER & TRAUTMAN 1948).

Most of these authors (LAMSON; EBERT & STEAD, KALTREIDER & MENEELY; HAHN, BALE & BONNER) suggest that exercise and adrenalin will cause a redistribution of blood corpuscles and plasma with a subsequent rise of the venous hematocrit, but no real increase in the volume of erythrocytes and plasma.

By means of the radio-iron method, GIBSON et al. (1946) observed that the spleen of abruptly killed animals contains less than 5 per cent of the total of blood corpuscles.

Further, it has been demonstrated that the particular technique used for blood depot investigations on animals may account for the variations in the results, and also that misleading findings may originate in an anesthesia (SJÖSTRAND, 1935, HAHN, BALE & BONNER, 1943).

The variation in the amount of blood in different venous systems has been shown to be affected by orthostatic conditions (ASMUSSEN, CHRISTENSEN & SJÖSTRAND, 1939, SJÖSTRAND, 1941, NYLIN & PANNIER, 1947).

On the basis of comprehensive investigations SJÖSTRAND, (1935, 1949, 1950) concludes that the pulmonary venous system plays an important part as a variable blood depot. This amount of blood is not, however, separated from the circulation but constitutes an important regulating factor (SJÖSTRAND 1950).

Still, judging from the forementioned investigations by GIBSON et al. (1946), and others, it can apparently be concluded that in normals all the blood corpuscles are in active circulation.

The principle of blood distribution will, therefore, seem more justified than the conception of blood depots in its original form.

CHAPTER II

Cell, plasma and blood volume in cardiac insufficiency

Investigations in cases of cardiac insufficiency have mainly been carried out with dyes, with a view to determining, in the first place, the plasma volume from which conclusions have been drawn regarding the total red cell volume and above all the blood volume.

In all earlier investigations red dyes as vital red, congo red and trypan red (KEITH, ROWNTREE & GERAGHTY, 1921; BOCK, 1921; BROWN & ROWNTREE, 1925; WOLLHEIM, 1931; UHLENBRUCK & VOGELS, 1931; MIES, 1931; GOLDBLOOM & LIBIN, 1935; EHRSTRÖM, 1936; OTT, 1939) are used. The determination of the plasma volume is based only on one or two blood samples withdrawn within 7 minutes after injection, which was considered sufficient time for mixing in the blood stream. Nor has any extrapolation been resorted to in these instances.

GIBSON & EVANS (1937) gave an account of their investigations with T-1824 which is determined by spectrophotometer and where the concentration is traced in the blood for up to one hour. These authors, who also introduced extrapolation for determination of the blood concentration, expressed the opinion that mixing with the dye takes place after 7.5 minutes in normal cases, and after 15—20 minutes in cases with cardiac insufficiency. Therefore, having established that the mixing time is prolonged in decompensated cases with the dye method, they pointed out that plasma volumes, based on values obtained before mixing, may yield values that are as much as 20 per cent too low. By means of the Gibson-Evans' method the plasma-, cell- and total blood volumes have been estimated in cases of cardiac insufficiency (GIBSON & EVANS, 1937; GIBSON, HARRIS & SWIGERT, 1939; HARRIS & GIBSON, 1939; WALLER, BLUMGART & VOLK, 1940; MENEELY & KALTREIDER, 1943; PERERA, 1945).

Another group of investigations were concerned firstly with studies of the variations in the total *Hb* and, secondarily, with the total amount of blood in cardiac insufficiency. These determinations were performed with the *CO*-method (PLESCH, 1922; SCHÜRMEYER, 1928; HITZENBERGER & TUCHFELD, 1929; EWIG & HINSBERG, 1930; STEINMANN, 1950).

PLESCH is the only investigator to determine primarily the total *Hb* content by means of the *CO*-method and to give any figures about total *Hb* content in congestive heart failure. He usually found it to have increased.

The forementioned investigations, with different methods, prove that in decompensation the total blood volume is increased. An increased blood volume is chiefly observed in mitral valvular defects and in congestive cor pulmonale. Contradictory results are obtained with regard to the total blood volume in cardiosclerotic and hypertensive heart diseases. However, the blood volume in right heart failure is indicated as greater than in left heart failure. These views are in agreement with results found at autopsy (ASCHOFF, 1930).

When compensation occurs, the blood volume usually decreases, but contradictory results have also been obtained.

Only a few authors have calculated the cell volume by means of the dye-hematocrit method (GOLDBLOOM & LIBIN, 1935; GIBSON & EVANS, 1937; OTT, 1939; HARRIS & GIBSON, 1939; WALLER, BLUMGART & VOLK, 1940; MENEELY & KALTREIDER, 1943). They observed increased values in decompensation. In few cases studied they found that the cell volume commonly decreases when compensation sets in.

In 1947 NYLIN & HEDLUND published investigations on the total red cell volume in 7 cases of cardiac decompensation estimated with the P^{32} method. In some cases an increase in corpuscular weight in decompensation in comparison with normal cases was demonstrated. In some cases, restoration of compensation was accompanied by a reduction in the total amount of erythrocytes. Compensated cases as a group were found to have about the same total red cell volume as normal cases. A slower circulation in decompensation was demonstrated.

A preliminary report from investigations on a larger material of heart decompensated cases was given by HEDLUND (1950, 1951).

Using the P^{32} -method and the venous hematocrit, ROOS, BAKER & FREIS (1950) established that there was no significant increase in the circulating blood volume in patients with congestive heart failure over controls.

From investigations with the P^{32} -method in 27 patients in congestive heart failure, PRENTICE, BERLIN, HYDE, PARSONS, LAWRENCE & PORT (1951) concluded, that the total red cell volume was markedly increased in cases with rheumatic valvular heart diseases but only slightly increased or normal in those with hypertensive cardiovascular or arteriosclerotic heart diseases. In 5 of 6 instances studied, they found that the blood volume (estimated by the P^{32} -method and the venous hematocrit) increased when compensation occurred.

More detailed analyses on the total red cell volume in congestive heart failure as well as changes in the volume at restored compensation have not yet been performed.

As to the cause of the increased blood volume, and of the amount of blood corpuscles, in cases of cardiac insufficiency, opinions differ. EWIG & HINSBERG and WOLLHEIM ascribe the increase in the blood volume in decompensation to a depot function that has ceased to operate and that, consequently, all the blood is to be found in the circulation. The administration of digitalis would allow the depots to function and thereby diminish the circulating amount of blood. BOHNENKAMPF explains the increased blood volume from an evacuation of the blood depots. EPPINGER and GOLDBLOOM & LIBIN

are of the opinion that, in cases of cardiac insufficiency, the depots are filled with blood to such an extent that no further blood can be stored and that, accordingly, the circulating blood volume is raised.

The increased amount of blood in decompensation is either a compensatory factor in the sense of conveying an adequate amount of oxygen to the tissue (SCHÜRMEYER, PLESCH, GOLDBLOOM & LIBIN) or it serves the purpose of adaptation to a slowed-down circulation (SCHÜRMEYER). HITZENBERGER & TUCHFELD, on the contrary, noted a diminution of the amount of blood in cases of insufficiency of the left heart, and this must, in their opinion, be a compensatory factor corresponding to the loss of strength of the heart.

EHRSTRÖM, WALLER & BLUMGART, MENEELY & KALTREIDER as well as HEDLUND (1951) attribute the rise in the amount of blood corpuscles to an increased erythropoiesis, stimulated by the anoxia.

CHAPTER III

Some erythropoietic problems

BLOOD CELL FORMATION

In the adult the medullary cavities of bones contain two types of marrow tissue: The hematopoietic portion — or the red, cellular marrow in which blood cells are formed; and the fatty or yellow marrow. In the fetus at the time of birth the entire marrow cavity of all bones is filled with red, cellular marrow; with advancing age this is replaced by yellow marrow, which appears first in the centres of the long bones and progresses distally and proximally from these points. Replacement occurs first in the more distal bones. The fatty marrow gradually increases in extent until at about the age of twenty years all the marrow of the long bones is of this type except that in the proximal ends of the femurs and humeri. Red marrow persists in the flat bones, hence hematopoiesis is most active in the ribs, sternum, vertebral bodies, pelvis and skull.

The fatty marrow consists of fat cells, blood vessels and a minimal amount reticular framework. Although blood cells are not formed in this portion of the marrow, there is ample room for expansion of the red marrow into the shafts of the long bones, and under proper stimulation fatty marrow may be replaced by hematopoietic marrow. Active marrow in the adult is estimated at from 3.4—5.9% of the body weight and “has an enormous functional capacity as well as considerable room for expansion” (WHITBY & BRITTON).

The normal form of erythropoiesis in the adult gives rise to the normal erythrocyte (the normocyte) by a process best referred to as normoblastic erythropoiesis. The most generally employed nomenclature for the classification of the maturation phases during the development of the red blood cells is based on the stainability of the cytoplasm with acid and basic stains (HELLY, 1910, MAXIMOV, 1927).

Directly from reticular cells (ROHR, 1940), or from more differentiated multipotent hemocytoblasts (FERRATA & SORTI, 1948) the first cell is formed for the erythropoiesis:

- 1) Pro-erythroblast (SCHULTEN, 1937; FERRATA & SORTI, 1948) [megaloblast (Sabin)]. A large cell with basophile cytoplasm and a nucleus provided with nucleoli.

The continued development is characterized by a diminished size of the blood corpuscles, decreasing and ultimately disappearing “visible” nucleus as well as by

a hemoglobin accumulation. This process is continuous, though three stages are to be distinguished (FERRATA & SORTI, 1948; TURNBULL, 1936).

- 2) Basophile erythroblast [early erythroblast, (Sabin)].
- 3) Polychromatic erythroblast in which the appearance of hemoglobin can be observed.
- 4) Orthochromatic erythroblast with entirely acidophilic cytoplasm.

NAEGELI (1931) has used the term macroblast to refer to the younger and larger erythroblasts, particularly in embryonal organs and in the marrow in states of rapid blood regeneration.

The cellular development of the erythrocytes is assumed to proceed by mitoses. There has been some discussion regarding the question whether the normoblast nucleus is discharged or dissolved intracellularly. On the other hand, WĄJDA and DORAN-JORDA, EMMELS, PLUM (1947) and BOSTRÖM (1948) believe that several erythrocytes may be derived from each normoblast by budding-off (gemmation) from the cytoplasm.

There has been some discussion as to whether, in the process of erythropoiesis, erythrocytes are formed from the polychromatic (BOSTRÖM, 1948, NORDENSON 1951) or orthochromatic stage (WINTROBE, ROHR, SCHULTEN and others), partly depending on the definition of the erythroblasts and on the nomenclature.

It is generally considered that reticulocytes are direct successors of ripe normoblasts after loss of the nuclei. Numerous investigations favour the conception that the reticulocytes are younger cells than the circulating erythrocytes.

Characteristic of reticulocytes is their ability to react supravitaly with certain basic dyes. Their basophilic material seems to represent the last remnants of the basophilic ribonucleoproteins present in the primitive cells from which the reticulocyte has been derived.

The reticulocytes are a little larger in diameter than the adult erythrocytes (DAMESHEK WINTROBE) and thinner (SUESS, LIMENTANI, DAMESHEK & DOLOFF, 1948). Another difference is that reticulocytes contain more protoporphyrin than do the adult corpuscles. Reticulocytes as well as polychromatic erythroblasts have an aerobic metabolism (JACOBSEN & PLUM, 1944 and others).

Studies of bone marrow in cardiac insufficiency.

Certain investigations of cases of cardiac insufficiency suggest an increased activity in the bone marrow (EPPINGER and others). ASKANAZY noted an increase of functioning bone marrow in the long tubal bones (i.e. the lower third part of the anticrus), and NORDMEYER (1931) observed an increase of the red marrow in the femur.

A hyperplastic bone marrow was ascertained in 2 cases of congestive heart failure (FRANK & HARTMANN 1931), in one case of secondary polycythemia due to cardiac and pulmonary disease (DAMESHEK, 1935), in 4 out of 22 cases of cardiac insufficiency (MICHELAZZI 1937) and in 15 out of 18 decompensated cases of valvular and hypertensive heart diseases (OTT 1939). A red active bone marrow was observed particularly

in decompensated mitral defects (WEIL 1901, MACHEY 1907, ZADEK 1927) as well as in congenital heart diseases (Weil 1901, MACHEY 1907, TODTENHAUPT 1927). BRAUN (1951) ascertained a hyperplastic bone marrow in 16 cases of cor pulmonale with venous congestion and cyanosis.

On the other hand HIRSCHFELD failed to notice any hyperplasia in the bone marrow in congestive heart failure with a moderate hyperglobulia. MICHELAZZI (1932) emphasized that he often observed hypofunction of the bone marrow in decompensated cases, without finding any relation to the age of the patients. He, accordingly, suggests that a slight congestion stimulates the activity of the bone marrow, while a chronic congestion may damage the bone marrow with hypofunction as a consequence.

The hyperplasia of the bone marrow is characterized by an increase of the normoblasts, sometimes observed in clusters (ASKANAZY, ZADEK, FRANK & HARTMANN, DAMESHEK, MICHELAZZI, OTT, BRAUN). OTT and BRAUN have pointed out that the increased erythropoiesis originated, in the first place, in an increase of the mature cell elements. The immature ones, on the other hand, failed to disclose any percentage increase.

Numerous mitoses have been encountered by BRAUN in some of his cases of congestive cor pulmonale, and by FRANK & HARTMANN in their two cases.

From the investigations by FRANK & HARTMANN and BRAUN, it seems as though the hyperplasia of the bone marrow was only connected with an increased erythropoiesis, as they have not observed any particularly marked participation of myeloid elements.

Findings of extramedullary hematopoiesis have been described in a few cases of cardiac disease (MEYER & HEINECKE 1905; WINTROBE 1936 and others).

MICHELAZZI (1937) made an attempt to compare the findings of marrow in cardiac insufficiency with the account of erythrocytes and hemoglobin in the peripheral blood, without tracing any relationship. In 13 of the cases of cardiac insufficiency in which the marrow had been examined OTT, at the same time determined the total red cell volume with the dye-hematocrit method without obtaining any relationship between the observed volumes and the findings of bone marrow suggestive of a rise in its activity.

When compensation had occurred, OTT subjected 7 of his cases to sternal puncture. In 6 of these cases he found a decrease in the number of nucleated red cell elements with a remission in the erythropoiesis. In one observed case of compensated valvular defect, NORDMEYER (1931) noted a normal distribution of red and yellow marrow in the femur.

In secondary polycythemia at high altitudes, ZADEK, SCHÖNHOLZER & LÜTHI (1944) and MERINO & REYNAFARJE (1949) observed the same type of regeneration, with increase in the mature cell elements.

RETICULOCYTES IN PERIPHERAL BLOOD

Data of the number of reticulocytes under normal conditions in man vary from 1 per mille to 18 per mille of the total of red blood corpuscles (PRICE-JONES, VAUGHAN & GODDARD, 1935, and others). From a very large material, TRACHTENBERG (1932) stated the limits to be from 3.0 to 14.5 per mille.

A sudden onset of increased erythropoiesis is accompanied by a considerable reticulocytosis. Reticulocytosis due to liver remedies or in spontaneous remissions in pernicious anemia, and during iron treatment of hypochromic anemia, are well known. Reticulocytosis occurs at the regeneration after hemorrhagic anemia. It has also been observed in conditions of increased destruction of erythrocytes.

MINOT & CASTLE (1935) assume that the reticulocytosis following anemia is in reality a physiological response to the lowered oxygen tension produced by the anemia.

A moderate bone marrow anoxemia has further been suggested as the cause of the reticulocytosis in some conditions such as reticulocytosis at high altitudes (BARCROFT et al., 1923; SEYFARTH, 1927; TALBOTT & DILL, 1936; SCHÖNHOLZER & LÜTHI 1944; HURTADO, MERINO & DELGADO 1945), the reticulocytosis in low pressure chambers (GOLDBLOOM & GOTTLIEB 1929; GORDON & KLEINBERG 1937; LÖWENHAUPT 1941; SAATHOFF 1950), the reticulocytosis in foetal life (FRIEDLANDER & WIEDEMER 1925; SEYFARTH 1927; KRAFKA 1930; SHAPIRO & BASSEN 1941), the reticulocytosis at acute respiratory insufficiency in the application of a pneumothorax (RISKA 1950), and the reticulocytosis in cases of tetralogy of Fallot (JOSEPHS 1950). It is only after a certain period of latency that the reticulocyte count rises; with increased intensity of stimulation the period of latency decreases (SAATHOFF 1950).

In 10 cases of cardiac insufficiency, EHRSTRÖM (1936) showed that during decompensation the number of reticulocytes increases (to 1.2–1.5 per cent). After compensation, the number is reduced (to about 0.5 per cent). The reticulocytosis may, according to him, be a manifestation of an increased new-formation, caused by the anoxia.

In 4 decompensated patients with cardiac insufficiency, WALLER, BLUMGART & VOLK (1940) observed a reticulocytosis of 1.2–2.7 per cent. During recovery, it fell to 0.5 per cent or less. Also the forementioned authors interpret the reticulocytosis as a sign of active erythrocyte production.

C. PLUM and R. PLUM have been able to show that the degree of reticulocytosis in the peripheral blood is also determined by the content of a ripening substance. This substance occurs principally in plasma, but also in the spleen, liver and red bone marrow. Generally, the content of ripening substances in the plasma of normal adults seems to vary inversely with the reticulocyte count. Still, the reticulocytosis, which is connected with an increase in the amount of blood corpuscles, is accompanied by a rise in ripening substances (C. PLUM 1943). In an attempt to explain this, JACOBSEN & C. PLUM (1943) state that at the early ages immature corpuscles react more easily to humoral stimulation.

CHRISTENSEN and C. PLUM (1947) assumed that the thyroid gland produces substances that are of importance to an activation of the ripening substances in the body, and that this function is regulated by the hypophysis.

REGULATION OF ERYTHROPOIESIS

Under normal conditions, the daily loss of blood corpuscles is made up for by new-formation. Many hematopoietic factors contribute to the creation of the mechanism that controls the erythropoiesis, adapting it to the requirements of the body. Mention should

be made here of metals, such as iron and, to a certain extent, copper and cobalt, amino acids, vitamins, and nutrients in the general sense, above all protein.

Also endocrine glands control, to some degree, the erythropoiesis. GORDON & CHARPPIER (1947), DAUGHADAY, WILLIAMS & DALAND (1948) and others have published general surveys on this subject. A moderate anemia and a hypoplastic bone marrow has been seen in hypothyroidism (BOMFORD 1938, and others) and after hypophysectomy (CRAFTS 1946, and others), as well as after thyroidectomy. A decrease in the red blood cell volume after hypophysectomy has been shown to occur by the P^{32} tagged cell method (BERLIN, VAN DYKE, SIRI, WILLIAMS, 1950). Hyperthyroidism and hyperaction of the cortex of the adrenal gland as well as various diseases affecting the hypophyseal-hypothalamic area have been observed to cause symptomatic polycythaemia.

Some authors (WINTROBE et al., and others) have noticed bone marrow stimulation following corticotrophin therapy, others could find no significant change (YOFFREY et al., 1951). A protracted administration of ACTH elevates the total circulating red cell volume, shown by the P^{32} tagging cell method, in normal rats (GARCIA, VAN DYKE, HUFF, ELMLINGER, ODA, 1951). WINTROBE et al. conclude that in diseases corticotrophin, instead of directly stimulating the bone marrow, in some way alters the process which has made the marrow function abnormally.

Further is to be mentioned, that in hypophysectomized subjects, a thyroid administration produces hyperplasia in the bone marrow. FINKELSTEIN et al. (1944) contended that male hormones stimulate an erythropoiesis.

A lowered oxygen tension has long been regarded as an important stimulus. Since BERT (1878) and VIAULT (1891) found polycythemia in animals and human beings exposed to low atmospheric pressure, many investigators have found that newcomers and residents at high altitudes exhibit a polycythemia, in general directly proportional to the degree, duration and continuity of the anoxia stimulus (SMITH et al.; DOUGLAS et al.; LICKNATZKY; CORDERO; ANDRESEN & MUGRAGE; FITZGERALD; MORALES; STAMMERS; HURTADO, MERINO & DELGADO; ROBLES GIL & TERAN; TALBOTT & DILL; WIESINGER and TOBLER; SCHÖNHOLZER & LÜTHI; MIESCHER, VERZÁR & VOEGTLI; VANOTTI and others). This polycythemic adaptation to high altitudes is reversible (HURTADO et al., and others).

A polycythemic reaction and a hyperactive bone marrow have been noted by several researchers (GREGG, LUTZ & SCHNEIDER 1919; CAMPBELL 1927; SABIN 1928; BOYCOTT & OAKLEY 1933; LOWENHAUPT 1941; WARREN 1941; DONNELL, JENSEN & ALT 1946) on animals in low pressure chambers.

While an anoxia may accordingly, exert a stimulating effect on the erythropoiesis, a surplus of oxygen should, supposedly, lower its activity. Such an affect has also been observed by oxygen administration at certain concentrations (CAMPBELL 1927; BOYCOTT & OAKLEY 1933; DONNEL, JENSEN & ALT 1946; TINSLEY, MOORE, DUBACH, MINNICH & GRINSTEIN 1949; COOPERBERG & SINGER 1951). By means of intravenous injections of oxygen DERSCA & VALTER (1926) succeeded in decreasing the number of erythrocytes in the blood in patients with anoxia.

However, investigations of bone marrow fragments on cultures *in vitro* have shown that the rate of proliferation of erythroblasts decreases as the atmospheric pressure is reduced (ROSIN & RACHMILEWITZ 1948; MAGNUSSEN 1949; ASTALDE, BERNARDELLI & REBAUDO 1951). On the other hand, ROSIN & RACHMILEWITZ found that 50% of oxygen stimulated the production and maturation of the blood corpuscles. MAGNUSSEN, on the contrary, observed the opposite effect of the same concentration of oxygen. He established the optimal production at the physiological oxygen tension. He has also assumed, that *pH* may have an influence on the cell production.

These discrepancies between the results may be explained from WARREN's investigations (1941). In rabbits kept in low pressure chambers, WARREN found a hyperplasia of the erythroid elements in the bone marrow which was characterized by a high rate of respiration in relation to the rate of glycolysis. The immature erythroid cells in the bone marrow should therefore have a metabolism of preponderantly oxidative type that would remain unaltered when the animals were exposed to low oxygen pressure. However, when WARREN exposed fragments from the bone marrow to a reduced oxygen tension, the rate of respiration was observed to abate while the glycolysis increased.

As there is proof that anoxic conditions can stimulate to increased erythropoietic activity, the question remains how this activation takes place. In principle, two general modes of procedure can be conceived, viz, either a direct effect on the bone marrow of reduced oxygen tension, or an effect on the bone marrow via some hormone-producing organs that has an activating influence on the erythropoiesis.

In support of the first-mentioned hypothesis, a lower oxygen saturation was observed in sternal punctates in cases of polycythemia secondary to cardiac or pulmonary disease (SCHWARTZ & STATS, 1949, BERK, BURCHENAL, WOOD & CASTLE, 1948) and in animals in low pressure chambers (GRANT & ROOT, 1947), being lower also than the simultaneously noted arterial oxygen saturation. The stimulating effect on the erythropoiesis observed by the administration of adrenalin and ephedrin has been explained by the fact that these remedies reduce the blood flow to the bone marrow and, consequently, also the supply of oxygen (DAVIS, 1941).

On the other hand, some investigations indicate that the anoxia can hardly have any direct effect on the erythropoiesis. GRANT & ROOT (1947) obtained the same degree of increased erythropoiesis when the loss of blood was caused by small successive bleedings without any diminution in the oxygen tension, as after a loss of 30% of the blood volume accompanied by lowered oxygen saturation in the blood of the bone marrow.

According to theories, advanced by CARNOT & DEFLANDRE (1906) and by MUELLER (1912) somewhere in the organism hypoxia is believed to produce specific factors that reach the bone marrow via the blood and stimulate erythropoiesis. By injecting in test animals serum from animals with induced hypoxemia, and observing the effect on the erythropoiesis, some authors have tried to ascertain the occurrence of the specific factors of hypoxia produced in the organism (WESTPHAL, 1944; BONSDORFF & JALAVISTO, 1949; and others). However, other investigators have not obtained any such result (FOERSTER, 1932; GORDON & DUBIN, 1934). REISSMANN (1950) who induced a chronic hypoxe-

mia in one of a pair of parabiotic rats and found stimulated erythropoiesis in both animals concluded that the stimulus was to be found in a humoral factor elicited by the hypoxemia in one of the two partners and then transferred to the other.

Inter alia, investigations showing that hypophysectomized animals are unable to respond by an increased erythropoiesis at anoxia (MEYER) speak in favour of the assumption that anoxia affects the erythropoiesis via some endocrine function. An increased weight of the adrenals has been ascertained by GARCIA et al. (1951) in normal rats treated with ACTH. Cortical hypertrophy during the administration of large doses of corticotrophin in human beings has also been demonstrated (O'DONNELL, FAJANS, WEINBAUM, 1951, SOKOLOFF, SHARP & KAUFMAN, 1951 and others). Increased adrenal weights were found by SUNDSTROEM & MICHAELIS (1942) and DARROW & SARASON (1944) in rats placed in low pressure chambers. Lastly, an increase in the size of the adrenal cortex with signs of hyperactivity has been noted in cardiovascular lesions (SELYE, 1947).

On the other hand, the removal of both adrenals produces a decrease of the O_2 uptake of the erythrocytes, while adreno-cortical extracts will restore the oxygen consumption of the erythrocytes (GONZALES & ANGERER, 1947). It seems fitting that a hypertrophy of the adrenal cortex takes place in conditions involving anoxia.

Thus, in all likelihood an interplay takes place between anoxia, endocrine organs and perhaps the central nervous system, stimulating the erythropoiesis. The possibility that the anoxia can directly exert a stimulating effect on the bone marrow is open to discussion.

LENGTH OF LIFE AND DESTRUCTION OF BLOOD CORPUSCLES

Several investigations on the life span of the red blood cells have been performed. The determinations have varied between 5 to 200 days, according to different methods. The lowest values derive from uncertain experimental conditions. With improved methods more agreeable values have been obtained.

From changes in the bile pigment in dogs, where a regeneration of the red cells had been induced by bleeding, HAWKINS & WHIPPLE (1928) calculated the life span of erythrocytes to 133 days. ASHBY (1921), WEARN, WARREN & AMES (1922), WIENER (1934), MOLLISON & YOUNG (1940), CALLENDER, POWELL & WITTS (1945), are agreed upon 120 days as an approach to an optimum length of life for transfused red cells as indicated by the method of differential agglutination. SHEMIN & RITTENBERG (1946) studied the change of isotope concentration with time of the hemoglobin haem of a subject fed ^{15}N -labelled glycine, which is a nitrogenous precursor of haem. From this the average life span of the erythrocytes was calculated to 127 days. JOPE (1946) determined the span of life of blood cells to 120 days by measuring the rate of disappearance of sulphaemoglobin from the blood of subjects who had been in contact with trinitrotoluene.

General surveys dealing with these problems are to be found in papers published by ASHBY (1948) and NEUBERGER (1951) among others.

Under pathologic conditions, as pernicious anemia and jaundice, the red blood corpuscles may be counted on having a shorter length of life (ASHBY and others). From the findings of hyperplastic bone-marrow and considerable reticulocytosis in some diseases, SCHIÖDT (1938) suggests that an increased production of erythrocytes correspond to a decreased duration of life.

The red cell amount is the resultant of two factors: the rate of blood production and the rate of blood destruction. Under normal conditions the daily destruction of red blood corpuscles is balanced by a daily production of the same size. However, the constant blood level may be maintained in two ways, according to SCHIÖDT (1938) who called these two possibilities the theory of longevity and the theory of destruction.

According to the forementioned theory the blood corpuscles have a definite lifetime. When they die of old age, they are intercepted by an organ of destruction which is assumed to act rather passively. According to the lastmentioned theory, the death of the erythrocytes is brought about by an active process. Each day a certain number of blood corpuscles will be destroyed, irrespective of their age. It is seen from these two theories that the age distribution curve will differ.

These are the extreme interpretations of the two theories. SCHIÖDT (1938) pointed out that the two theories may approach each other. It is then possible that the active destruction chiefly overtakes the older cells. Further, if the lifetime of different corpuscles under certain conditions is variable, some of the cells will die comparatively young, while others will grow older. Thus, the agedistribution of the corpuscles relating to the two theories will approach to one another.

When a regeneration of blood corpuscles takes place, increased production or decreased destruction, or both, will cause the rise of the erythrocytes.

CHAPTER IV

Other hematologic problems

DIAMETER OF RED BLOOD CORPUSCLES

Two fundamentally different methods have been used for determination of the diameter of blood corpuscles, as described below:

1. HALOMETRY (diffractometry) by means of which measurements have been made by YOUNG, PIJPER, SCHALM, BOCK, EVE, COX & PONDER, among others. However, the degree of anisocytosis cannot be determined by halometry, nor can the important PRICE-JONES distribution curve be procured.

2. *Direct measurements with microscope.* The earliest measurements were performed by VAN LEEUWENHOEK (1674), MANASSEIN (1871), BUNTZEN (1879), GRAM (1883), MALASSEZ (1889), PRICE-JONES (1910).

The technique has varied: a) *micrometry of wet preparations* (v. BOROS, JÖRGENSEN & WARBURG, MEULENGRACHT, GRAM, GORMSEN and others), b) *Microprojection of dry preparations* (MALASSEZ, PRICE-JONES, MOGENSEN, and others), c) *Micrometry of dry preparations* (PONDER & MILLER, and others).

Compilations of the diameter of normal blood corpuscles, determined by various methods have been published by MOGENSEN (1938), PONDER (1944) and G. LARSEN (1949), among others. The values vary, depending on the particular method employed.

PRICE-JONES (1920), PONDER & MILLER (1924), and others, found a greater cell diameter in wet preparations than in dried blood films. On the other hand, COLLATZ noted somewhat lower values in erythrocytes suspended in plasma than in dried preparations. Finally, many investigators (OHNO and GISEVIUS, HAMMERSTEN & STÄHLE, GOLDBERG, GUNVALL, HAMMARSTEN & LINDGREN among others) are of the opinion that the diameters determined in the wet and dry preparations are the same. An increase of the red cell diameter is obtained when oil immersion is used instead of the dry optical system. G. LARSEN, in his detailed studies, found that many of the observed differences are due to optical phenomena or inaccuracies in the preparation technique, such as dyeing without using a buffer solution. As a rule, most investigators are of the opinion that fixing and staining of the blood film do not alter the size of the cells.

Apart from studies of normal cases of different sex, age, race, the erythrocyte diameter has been determined in different morbid conditions. The literature at disposal is comprehensive.

Mean corpuscular diameter in congestive heart failure.

Also certain investigations of the size of the erythrocyte in cardiac insufficiency have been carried out. A compilation of the results is made in *table 2*. In the earlier investiga-

Table 2. Mean corpuscular diameter in congestive heart failure.
(Survey of literature).

Author	Diagnostic remarks	MCD	Commentaries	Used method
Vaquez (1895)	Chronic cyanosis	Increased diameter		
Aubertin	Chronic cyanosis	Increased diameter		
Price Jones (1921)	Congestive heart failure	7.22 μ (6.90–7.51) 15 cases	Correspond to normal values. 7.24 μ	Micrometri, dry prep., without immersion
Jørgensen S. & Warburg E. (1927)	Congestive heart failure	8.7; 8.6; 9.0 μ 3 cases	Normal value 7.7 μ	Micrometri, wet prep., immersion
Frank E. & Hartmann E. (1931)	Congestive heart failure (severe cases)	In 6 cases macrocytes and erythroblasts in peripheral blood. MCD determ. in 1 case (> 8.0 μ)	Generally only slight anisocytosis, in a few cases macrocytosis	
Malamos B. (1935)	Congestive heart failure	Slight macrocytosis in 3 of 8 cases	Normal value 7.4 μ	Abbe's drawing apparatus, dry prep., immersion
Ask-Upmark E. (1936)	Congestive heart failure	8.0 9.0 (number of cases are not noted)		
Luckner H. & Tölger F. (1936)	Congestive heart failure	1) 8.03; 8.00; 7.92 3 cases 2) macrocytosis in 5 of 36 cases	Normal values 7.46 μ (7.34–7.6 μ) Normal values 7.3–7.7 μ	Abbe's drawing apparatus, dry prep., immersion Diffactometri, dry prep., immersion
Schalm L. (1937)	Congestive heart failure	7.9; 8.1; 8.1; 8.4 μ 4 cases	Normal value 7.8 μ	Diffactometri
Mayer G. (1940)	Morbus cordis (No congestion)	7.60 μ (7.35–7.79 μ) 12 cases	Normal values 7.65 μ (7.4–8.0 μ)	Micrometri, wet prep.
Lindgren G. (1947)	Diseases of the circulatory system	2 of 25 cases reveal a MCD > 8.0 μ , in 1 of the 2 cases severe congestion	Normal values 7.68 μ (7.4–8.0 μ)	Micrometri, dry prep., immersion
Nylin G. & Hedlund S. (1947)	Congestive heart failure	8.0–8.61 μ in 13 of 20 cases, average MCD 8.20 μ	Normal values 7.68 μ (7.4–8.0 μ)	Micrometri, dry prep., immersion

tions, chronic cyanosis is stated to produce an increased *MCD* (VAQUEZ and AUBERTIN). Normal values of *MCD* were, on the other hand, found by PRICE-JONES in a major investigation series, as well as by MAYER. The latter, however, did not state whether his cases of heart disease are subjected to decompensation. PRICE-JONES observed macrocytosis in cases of pulmonary emphysema. In other investigations, the occurrence of macrocytosis in cardiac insufficiency has been ascertained (SCHALM, LINDGREN, ASK-UPMARK, JÖRGENSEN & WARBURG, FRANK and HARTMANN, MALAMOS, LUCKNER & TILGER). LUCKNER & TILGER found that macrocytosis is rather rare (in 5 out of 36 cases). LINDGREN confirms this, though he only states decompensation in 1 out of 25 cases. The other authors have dealt with small investigation series comprising but a few cases. NYLIN & HEDLUND (1947) ascertained a higher frequency of macrocytosis in cardiac insufficiency (in 13 out of 20 cases). A more detailed analysis on the basis of a larger material has since been presented by HEDLUND (1949 and 1950).

CABOT (1904), FRANK & HARTMANN and GROEN & GODFRIED (1945), in severe cases of cardiac insufficiency, observed erythroblasts in the peripheral blood.

The cause of macrocytosis.

Macrocytosis in hepatic affections is well known and has been well described. Comprehensive literary data are to be found in LARSEN's inquiry (1948). Owing to the fact that, in cardiac insufficiency, liver stasis with jaundice frequently occurs, the same interpretations of a macrocytosis have often been given as in hepatic diseases, though also with due regard paid to certain particular changes in the blood in cardiac diseases. Broadly speaking, two principal trends in the interpretation of the macrocytosis can be distinguished. One explains the origination of a macrocytosis from peripheral factors in the circulation, the other from changes in the erythropoiesis with a secondary discharge of macrocytes to the circulation.

Macrocytosis due to "peripheral disturbances".

In the earliest investigations of cases with heart failure, macrocytosis was supposed to be incidental to peripheral factors. The increased CO_2 content in the blood was, in particular, regarded in the decompensated cases as a cause of macrocytosis (MALAMOS, WIECHMANN & SCHÜRMEYER 1927), as well as in patients with pulmonary emphysema (PRICE-JONES 1924). Analogously, WIECHMANN & SCHÜRMEYER ascertain that the *MCD* was larger in venous than in arterial blood. Entirely contrary observations have, however, been made. Thus, MANASSEIN (1871) noted that the *MCD* decreased with an increasing content of CO_2 . In decompensated cases of mitral defects, MOSEL and EINHORN (1930) found a bigger *MCD* in arterial than in venous blood. Finally, WARBURG (1922), VAN SLYKE, WU & MACLEAN (1923) and DRYERRE, MILLAR & PONDER (1926) found that the CO_2 content in the blood hardly exerts any effect on the volume of erythrocytes.

Daily variations and changes in the *MCD* after exercise, which have been explained from variations in alkalinity, as well as in certain diseases with acidosis, have been

shown by LARSEN (1948) to be the result of the different staining qualities of the blood cells under different conditions. WARBURG and VAN SLYKE, WU and MACLEAN have urged that the changes in H^+ -concentration are too small, in vivo, to give rise to any changes in the volume of erythrocytes.

Macrocytosis, connected with hepatic affections, has come to be supposed to originate in the peripheral blood through the influence of bilirubin or bile acid salts (MEULENGRACHT & GORMSEN 1948). GRAM & MEULENGRACHT, LINDGREN have ascertained a correlation between the content of bilirubin and macrocytosis, while a great many authors (MALAMOS, SCHULTEN, MEULENGRACHT and GORMSEN, JÖRGENSEN & WARBURG, HAMMARSTEN, LARSEN and others) have failed to do so. It has further been known that a macrocytosis may appear in liver diseases without a jaundice (HOLLER & KUDELKA, HAMMARSTEN, LINDGREN) and that the macrocytosis may remain after the jaundice has disappeared (ROSENBERG, HAMMARSTEN and others). On the other hand, ENGELSEN (1884) stirred erythrocytes from healthy persons in jaundice serum, noting that they increased in diameter. GRAM and MEULENGRACHT could not reproduce these experiments. Further, LARSEN (1948) showed that this observed increase in *MCD* is not due to the icteric plasma as such, but that the increase is an artefact caused by technical manipulations in the course of the experiment.

Also osmotic disturbances, i.e. changes in the serum protein have been propounded as a cause of macrocytosis (GAMNA, ROSENBERG and others). However, LARSEN failed to find any correlation between the *MCD* and total protein or globulin concentration in plasma. BROCH found no increase in *MCD* when the concentration of electrolytes was reduced. LARSEN (1948) could trace no change in the *MCD* when the erythrocytes were stirred in decreasing concentrations of hypotonic salt solutions. On the other hand, a change in the size and diameter of the erythrocytes may, conceivably, take place, when osmotically active products accumulate within the cell. Such an active product is to be found in, e.g., lactic acid, which has been seen to form more rapidly in blood corpuscles than in plasma at stasis (TSAI CHIAO, CHEN & CHIU 1942/43).

Macrocytosis due to "central disturbances".

In addition to macrocytosis, anisocytosis, polychromasia and reticulocytosis have been observed in liver diseases (ROSENBERG, HAMMARSTEN, LARSEN, and others), in experimentally produced hepatic fibrosis (GOLDBERG, HAMMARSTEN, HELLSTRÖM, LINDGREN and PLUM 1950), as well as in cardiac insufficiency (LUCKNER & TILGER, FRANK & HARTMANN), and under anoxic conditions at high altitudes.

These changes have been interpreted as an expression of a changed erythropoiesis of a macronormoblastic type with secondary discharge of macrocytes to the peripheral blood. Changes in the bone marrow of precisely this type have been observed in liver diseases (BLEICHRODER & LEVEY; BARMAN, AXELROD, HORAN, JACOBSON, SHARP & VONDER-HEIDE; WINTROBE, and others) and heart diseases (EPPINGER; MICHELAZZI; OTT; FRANK & HARTMANN) as well as under anoxic conditions at high altitudes (SCHÖNHOLZER and LÜTHI).

By ascertaining that the Price-Jones' curve of the erythrocyte diameters is composed of at least two populations, one with an *MCD* of a normal size, the other with an enlarged *MCD*, the latter decreasing in size as the state of the liver disease improves, LARSEN (1948) has, likewise, upheld the conception of macrocytosis as of a central hematopoietic origination. A similar analysis of Price-Jones' curve has been made by MOGENSEN (1938) in liver diseases.

It will, accordingly, seem that the macrocytosis has a central genesis, originating in some change in the normoblastic erythropoiesis. Apparently, no definite evidence has been adduced for the alleged increase of the *MCD* of the erythrocytes in the circulation through the influence of peripheral factors.

If the theory of the macrocytosis as resulting from a changed erythropoiesis be accepted, the question of the cause of this change remains.

In the opinion of LUCKNER & TILGER (1936), the macrocytic erythropoiesis in cardiac insufficiency depends on a simultaneous hepatic dysfunction. ASK-UPMARK (1936) points out that it is possible, though by no means certain, that the macrocytosis, can be attributed to the hepatic affection. He even suggests the possibility of a toxic effect on a hematopoietic centre in the diencephalon. FRANK and HARTMANN (1931) found macrocytosis only in some cases of cardiac insufficiency. They discuss whether it can be due to the venous stasis in cases of heart failure with a severe right heart insufficiency or to the simultaneous hepatic changes.

There is, apparently, justification for an elucidation of this connection by referring, in the first place, to investigations dealing with the causes of macrocytosis in hepatic diseases when a hematopoietic genesis has been attributed to it.

The occurrence of macrocytosis and anisocytosis in hepatic diseases has been interpreted by VAN DAYN, HIJMANS VAN DEN BERGH, ROSENBERG & WALTERS, WINTROBE & SCHUMACHER as a manifestation of an incapacity of the injured liver to store or to utilize the antianemic principle of pernicious anemia. GOLDHAMAR, ISAACS & STURGIS (1934) concluded that an injured liver is able to store the active principle but that the liver is "unable to release the necessary substance for red cell development". SCHIFF, RICH & SIMON (1938) prepared liver extracts from two cases with portal cirrhosis and one with chronic passive congestion, by means of which they obtained a remission in pernicious anemia. In their view the liver could store the hemopoietic principle.

Considering that the anemia in hepatic cirrhosis is not megaloblastic but macronormoblastic, HUANG & WANG (1949) contended that no disturbance occurs either in the storing or in the synthesis of the hemopoietic principle. LINDGREN agreed in this view.

WINTROBE (1936) has noted favourable effects of intramuscular liver therapy in several cases of macrocytic anemia in hepatic diseases, while ROSENBERG & WALTERS (1936), HIMSWORTH (1947), BRAIER (1948), LARSEN (1948), JARROLD & VILTER (1949), HUANG & WANG (1949) have failed to observe any such effects. Nor have GOLDBERG et al. (1950) done so in experimentally produced liver fibrosis in animals. Also, BRAIER (1948), LARSEN (1948), and JARROLD & VILTER (1949) failed to find any reaction to folic acid in macrocytic anemia in hepatic cirrhosis. Nor could any definite effect of iron (BRAIER,

1948; HIMSWORTH, 1942) or of blood transfusions (BRAIER, 1948) or of excess of dietary protein (HIMSWORTH 1942) be observed.

The influence of vitamins has been studied i.a. by GOODHART, VAUGHAN & WILLS, who noted improvement in macrocytic anemias after administration of yeast. LARSEN (1948) reported a decrease of the macrocytosis in some cases of liver diseases after treatment with niacin, but failed to notice any effect from aneurin and lactoflavin. On the other hand, CARTWRIGHT (1947) in a synoptic work states that no lack of nicotinic acid has been noted in man and that no evidence has been forthcoming for any indispensability of riboflavin for a normal erythropoiesis in man.

On the other hand, some other hepatic function may be disturbed from an injury to the liver, to such an extent as to affect the erythropoiesis, resulting in a macrocytosis. In acute cases of hepatitis, LINDGREN (1949) infers that "it seems probable that the mean diameter and the excretory, secretory and metabolic functions of the liver are in some way connected". However, BRAIER (1948) failed to affect a macrocytic anemia in liver cirrhosis by cholin and methionin.

To mention is also that in the opinion of HUANG & WANG (1949) and BERMAN et al. (1949), the splenic stasis in cases of liver cirrhosis will cause an increased hemolysis, stimulating the bone marrow into a more marked activity that will produce macrocytes.

GOLDBERG, HAMMARSTEN, HELLSTRÖM, LINDGREN & PLUM (1950) in experimental liver fibrosis found a macrocystosis, but a lower increase in the *MCD* when — after the ligation of ductus choledochus — the animals got a daily injection of splenic extract or ACTH.

OSMOTIC RESISTANCE OF RED CORPUSCLES

According to PONDER (1936), the resistance of the erythrocyte may be dependent on at least four factors, viz,

- 1) its water content,
- 2) its inner osmotic pressure,
- 3) the critical volume that it is capable of holding, i.e. the maximal cell volume to which the cell may remain intact, and
- 4) the degree to which it acts as a perfect osmometer, impermeable to the osmotically active substances which it contains.

The water content of the cells and the inner osmotic pressure are, in PONDER's opinion, generally of secondary significance. The critical volume to which the erythrocyte may attain is due in principle to the cell type, while the degree to which the blood corpuscle acts as a perfect osmometer depends on the nature of the hypotonic environment. Temperature, *pH*, salt concentration, amount of glucose in surrounding media may change the osmotic resistance (PARPART, LORENZ, PARPART, GREGG, CHASE 1946, TSAI, CHEN, CHIU 1942, et al.), as well as the occurrence of bile acids (LINTZEL, and others) and lysolecithin formation (BERGENHEM 1938, FÄHRAEUS), and the accumulation of osmotically active substances within the cell, e.g., lactic acid (TSAI, CHEN and CHIU

1942). When CO_2 -gas is made to pass through the blood, the resistance of the blood corpuscles is reduced (VAN SLYKE and CULLEN) while increasing when O_2 is introduced (BUTLER). SIMMEL (1925) however considers the variations in CO_2 -tension and O_2 -tension *in vivo* too small to produce any changes in the resistance.

When the resistance of the blood corpuscles is determined by placing them in a hypotonic sodium chloride solution, all four variables mentioned by PONDER will be measured at the same time. However, the measurement of osmotic resistance, including data regarding incipient and total resistance, is worth while and may offer some information since any given population of red cells possesses individual cells which differ from one another in the critical volume at which hemoglobin is permitted to diffuse out. CASTLE and DALAND (1937) consider that the difference in the resistance of blood corpuscles to hemolysis in a hypotonic sodium chloride solution is more due to differences in form than in osmotic behaviour.

DAMESHEK and SCHWARTZ (1938) noticed a fair correlation between spherocytosis and decreased resistance, while the resistance increases when the cell diameter grows larger and the thickness is reduced.

In normals, SUSS, LIMENTANI, DAMESHEK & DOLOFF (1948) express the opinion that it is the age of the erythrocytes that decides the varying degree of hemolysis. The oldest ones, which are the most spherocytic, are first hemolyzed. On the other hand, the age of the subject between 5—84 years should not influence the resistance of the blood corpuscles (WIECZOROWSKI and FISHBACK 1941).

Several investigators (NORWITZ & PRATT 1908, HAMI & PRATT 1909, SATTLER 1910, SNAPPER 1912, HANDOWSKY 1912, SIMMEL 1925, ODA 1927) have demonstrated how the osmotic resistance grows at regenerative blood formation owing to the introduction into the blood stream of younger erythrocytes with increased resistance. In aplastic anemia where the disintegration exceeds the regeneration a reduced resistance is observed (SIMMEL 1925).

After some time spent at high altitudes, the test subjects have disclosed an increased resistance parallel with an increased erythrocyte count and diameter of the blood corpuscles, viz, cells that were regarded as newly formed (WILBRANDT & HERRMANN 1944, SCHÖNHOLZER & LÜTHI 1944, VANNOTTI & MARKWALDER 1939).

In liver diseases, LARSEN (1949) ascertained an increased maximal resistance which is correlated to the size of the larger blood corpuscle population calculated by Larsen from the distribution Price-Jones' curve.

Resistance of red blood corpuscles in congestive heart failure.

In cardiac insufficiency, the state of resistance of the blood corpuscles during decompensation has been subjected to some inquiry. Nevertheless, information is sparse as to the changes in the resistance brought about by the treatment of decompensation.

A reduced minimal resistance in decompensated heart failure was observed by MELDOLESI (1931), by BUCCIANI & ROSSI (1934) in investigations on 33 cases, and by FRAN-

CESCON (1936) in 8 cases, as well as by WALLER, BLUMGART & VOLK (1940) in 4 cases. Simultaneously an increased maximal resistance was obtained by MELDOLESI, BUCCIANTI & ROSSI and FRANCESCON. GREENTHAL & O'DONNELL (1921), however, ascertained both a reduced minimal and maximal resistance in 3 cases of heart failure with cyanosis. At compensation FRANCESCON (3 cases) and WALLER et al. (4 cases) noticed an increase of the minimal resistance and FRANCESCON a decrease of the maximal resistance.

The prerequisites for a reduced minimal resistance in cardiac insufficiency may be several. It was attributed to physico-chemical changes in the peripheral vascular system (BUCCIANTI & ROSSI) or changed tissue metabolism (WALLER) due to the stasis.

TSAI, CHEN & CHIU (1942) ascertained in artificially produced stasis a reduced resistance and a swelling of the erythrocytes to spherocytes. They explained this as due to an accumulation of osmotically active substances in the cell. They also maintained that the stasis changes the permeability of the cellular membrane, having observed that the K content in the plasma increases with the duration of the stasis.

WALLER (1939) further conducted oxygen through the blood but found that neither a low O_2 -tension nor a high CO_2 -tension could bring about the reduced resistance. SIMMEL (1925) was of the opinion that the changes in the CO_2 and O_2 tensions were too small in cardiac stasis to produce any variations in resistance in vivo.

In addition, the hemocatonistic effect or sensibilization to hemolysis caused by the spleen must be further considered (EPPINGER 1920, FÅHRAEUS 1924, ORAHOVATS 1926, BARCROFT 1936, MELLGREN 1939, and others) involving a reduced osmotic resistance. This explanation has been adopted by FRANCESCON on his observation of decreased minimal resistance in congestive heart failure.

The lysolecithin formation in the plasma is according to BERGENHEM & FÅHRAEUS (1936) accelerated by the separation of blood corpuscles from the plasma. This process occurs in vivo in the spleen (FÅHRAEUS) through its mechanism for separating blood corpuscles and plasma. KNISELY (1936) maintains that the erythrocytes are retained in the splenic sinus while the plasma is filtered out into the spleen pulp and taken off with the efferent vein. Sinus empties its content of blood corpuscles into the veins after a certain space of time.

In cases of liver cirrhosis and chronic cardiac congestion, S. E. BJÖRKMAN (1947) found structural changes of the spleen sinus wall, resulting in a tightening of the sinus filter. He attributes this effect to the portal hypertension which also gives rise to the dilatation of the sinus. "Tightenings" of the structure counteract the increase of the stroma breadth which follows on sinus dilatation.

BUCCIANTI and ROSSI as well as FRANCESCON suggest the increased maximal resistance in congestive heart failure to be an effect of regenerative erythrocyte formation.

SERUM IRON

The total body iron is about 4–5 gm in normal human beings. 60–75 per cent of this is included in the hemoglobin of the blood corpuscles. 3.0–5.0 per cent being traceable in the myoglobin. About 15 per cent is stored as ferritin in liver, spleen and marrow. Other known iron constituents constitute less than 1 per cent of the total body iron. (according to GRANICK 1949).

In blood plasma, non-heme iron compounds occur as ferrous iron and as siderophilin, a protein which is normally only $\frac{1}{3}$ saturated with iron.

The plasma iron is influenced by 1) the quantity of iron absorbed from the intestine, 2) the adequacy of the tissue iron reserves, 3) the capacity of the bone marrow to utilize iron for hemoglobin synthesis and 4) the activity of hemolytic processes.

The iron bound in hemoglobin is fixed and incapable of exchange with plasma iron (HAHN, BALE, ROSS, HETTIG and WHIPPLE 1940). The iron liberated from disintegrating blood corpuscles is immediately utilized for the building-up of new hemoglobin (CRUZ, HAHN, BALE 1941; HAHN, BALE and BALFOUR 1941), even when plentiful iron reserves are available. They concluded that the iron liberated from the disintegrating blood cells is more rapidly utilized than the iron in the depots. Iron derived from hemoglobin of destroyed red blood cells was in part transformed into ferritin by the liver and spleen (HAHN, GRANICK, BALE and MICHAELIS, 1943, GRANICK and HAHN 1944).

Iron turnover studies have shown that about $\frac{2}{3}$ of the plasma iron turnover is an effect of the red cell iron turnover (HUFF, HENNESSY, AUSTIN, GARCIA, ROBERTS and LAWRENCE 1950). They also expected the liver iron turnover to be one tenth of the total plasma turnover. Their results also indicate that probably less than 1% of the total plasma iron turnover is concerned with absorption.

Of interest in the present investigation, is their ascertainment of increased plasma and red cell iron turnover values in cases of secondary polycythemia.

Thus, it seems reasonable to assume that serum iron is a good indicator of the balance between the building and destruction of erythrocytes.

The serum-iron level in different physiologic and hematologic diseases has been described in numerous monographs (HEILMEYER and PLÖTNER 1937, MOORE et al 1937, SKOUGE 1939, VAHLQUIST 1941, WALDENSTRÖM 1944, TÖTTERMANN 1949, VANNOTTI and DELACHAUX 1949, and others).

In experimentally produced hemolysis, as after phenyl-hydrazin administration, a transient and, in several instances, considerable increase of the serum iron has been observed (MOORE et al 1937, HEILMEYER and PLÖTNER 1937, SKOUGE 1939, VAHLQUIST 1941).

The effect of the anoxia on the iron metabolism has been studied in a few investigations. Thus, LINTZEL and RADEFF (1939) ascertained on rats, subjected to iron-free diet in a low pressure chamber, that the stored iron in the liver and spleen decreased while the hemoglobin iron increased. When the animals were reacclimatized, the hemoglobin iron was reduced while the stored iron increased. According to these two authors, only

a displacement of the iron occurred, since the sum of the organic iron and of the hemoglobin iron remained constant and no excretion of iron was ascertainable. On the other hand, VAHLQUIST (1941) demonstrated that the serum iron in reacclimatized animals placed in a low pressure chamber showed a transient reduction followed by a moderate increase. Similar variations in the serum iron were observed by VAHLQUIST (1941) in newborns.

VANNOTTI and MARKWALDER (1939), who studied serum iron at high altitudes, found an initial increase of serum iron as well as bilirubin, while simultaneously the blood corpuscle resistance and erythrocyte count diminished. During the subsequent blood cell regeneration, the serum iron and bilirubin decreased. When the subject had come back to the sea-level, the polycythemia again decreased simultaneously with an increase of the serum iron, though the bilirubin values remained normal.

The iron in different depot organs is also submitted to variations.

In an autopsy material of decompensated heart diseases, LUBARSCH (1927) and NORDMEYER (1931) observed that the iron quantity in the liver and spleen was much lower than normal. Nordmeyer supposed that the iron liberated from the organs was used for the hemoglobin formation, since the blood volume is increased at cardiac insufficiency.

Still, it is quite possible that an injured liver may be incapable of storing iron. Thus, in liver cirrhosis traces of ferritin may escape from the injured cells into the serum (GRANICK 1949).

Hypersideremia has also been ascertained in acute hepatitis (WARBURG and KREBS 1937, HEMMELER 1939, SKOUGE 1939, WALDENSTRÖM 1940, VAHLQUIST 1941, BJERRE and CHRISTOFFERSEN 1942, BRÖCKNER-MORTENSEN 1943, and others), and in some cases of liver cirrhosis (HOWARD 1950). The latter was not not verified by HEMMELER, BJERRE and CHRISTOFFERSEN, and BRÖCKNER-MORTENSEN. The reason for the hypersideremia was thought to lie in disturbances to the function of the liver. A deterioration of this function was demonstrated parallel with the hypersideremia (HOWARD 1950). Even when a hyperbilirubinemia is ascertained, this does not run parallel with the hypersideremia, as the serum iron values may be elevated even after the icterus has disappeared (SKOUGE 1939, HEMMELER 1939, VAHLQUIST 1941, BJERRE and CHRISTOFFERSEN). Thus, to judge from these investigations, it is not certain that the liver dysfunctions alone can account for the hypersideremia in the forementioned conditions.

The intestinal mucosa regulates the absorption of iron by forming ferritin. Further absorption is blocked until some of the initially absorbed iron has passed out into the blood stream. Reabsorption of iron is not, apparently, unfavourably affected by the anoxia, seeing that test animals who had had their iron depots emptied and were then placed in a low pressure chamber quickly increased their hemoglobin iron from the iron administered in the diet (LINTZEL and RADEFF 1930). GRANICK (1949) has likewise shown that a deficient oxygen supply to the intestinal mucosal cell causes a quick reduction of the trivalent iron of the ferritin molecule, a reaction resulting in a passage of iron into the blood stream. As far as the present author has been able to ascertain, no investigations exist concerning the question whether an edema in the intestinal mucosa

exerts any influence on the resorption. Through excretion, the body loses a maximum of 1.5 mg of iron daily (LINTZEL 1927, FARRER and GOLDHAMAR 1935, HAHN, BALE, KAMEN, HETTIG and WHIPPLE 1939, COPP et al 1943 and others). In normal conditions, only small amounts of iron are excreted with the bile (STRANSKY 1931, HAHN, BALE, KAMEN and WHIPPLE 1939, HAWKINS and HAHN 1944), while the pigment portion of the hemoglobin is excreted totally as bile pigment (HAWKINS and HAHN 1944).

An increased excretion of iron by the kidneys has been observed in morbid conditions with increased serum iron (KISCH, 1922, HEMMELER, 1939). KISCH suggests this as the effect of an insufficiency of the reticuloendothelial system. It is also postulated by CARTWRIGHT, HAMILTON, GUBLER, KUHN & FELLOWS (1950) that the level of plasma iron is regulated by the reticulo-endothelial system, and that the functional activity of its cells is influenced by the adrenal cortex.

Preliminary reports on the serum iron condition in cardiac insufficiency have been published by the present author (1949 and 1950).

Urobilinogen excretion.

The urobilinogen excretion in feces has been considered to be a favourable indicator of the blood cell destruction and breaking down of hemoglobin. The urobilinogen excretion in urine is too much influenced by the conditions of the liver cells to be an acceptable indicator of the blood cell destruction (ZOYA 1936, and others).

The urobilinogen excretion in feces and urine has been studied in a number of diseases, a few investigations being available also in the case of cardiac insufficiency.

WATSON did not perceive any increase of the urobilinogen excretion in feces in cardiac insufficiency. WEISS seems to note a tendency to an increase though without finding any distinct difference from the normal. WALLER and BLUMGART (1940), in 5 cases of cardiac insufficiency, observed a urobilinogen excretion in feces varying between normal and slightly elevated values. In 2 cases this increased when the blood volume decreased at the entrance of compensation. They did not find any definite correlation between the reduced cell volume and periods with an increased urobilinogen excretion. Simultaneously, they ascertained an increased excretion of urobilin in the urine during the decompensation which they attributed to the reduced excretory capacity of the liver through the stasis.

EHRSTRÖM (1936), who examined 10 cases of cardiac insufficiency, on the other hand maintained that the liver stasis was not the principal cause for the urobilinuria in decompensation. He also found that the urobilinuria was directly proportionate to the urobilinogen excretion in feces and concluded that the increased total excretion of urobilin, when the symptoms of insufficiency were subsiding, is a manifestation of increased hemolysis. In all of Ehrström's 10 cases, urobilinuria occurred during the observation period, which exceeded the normal value.

CHAPTER V

Methods of study

THE P^{32} -METHOD

Apparatus and measurement technique.

$^{15}P^{32}$ which is one of the isotopes of phosphorus that can be produced by nuclear excitation, is a negatron emitter (β -radiation) of half-life 14.3 days, with a disintegration rate of 4.8% per day.

The operation suit for measuring the radiation is composed of a Geiger-Müller counter connected to a recording apparatus which consists of different electronic circuits, scaling circuit and a mechanical recorder. The apparatus is also provided with a well-stabilized high voltage device.

Most of the present investigations are performed either with an equipment from L.K.B., Stockholm (scale-of-100) or with an equipment from Industrial Products Lt. U.S.A. (scale-of-64).

Mica window, bell type β -ray G.M. tubes were used throughout for the measurements. Mostly tubes from the Victoreen Co., U.S.A. have been used.

Details of the technical arrangements will be found in every text book dealing with radioactive isotopes.

When measuring radioactivity the characteristics of the G.M.-tube have to be ascertained, background counts determined and resolving capacity of the apparatus calculated. To establish the operating voltage of a tube, the number of impulses from a standard specimen is registered as a function of the anode voltage. The curve, thus obtained, reveals a plateau, where the number of impulses is practically independent of small fluctuations in the voltage. The measurements are to be made on this part of the curve, which should have a good length and only a slight slope. Daily determinations of the operating voltage of the G.M.-tube by a uran-standard have been performed, also implying a control of the reliability of the recorder equipment.

Background counts due to radiation originating in the surroundings and cosmic rays can be reduced by proper lead shielding of the chamber. This shielding is important also in view of the fact that G.M. tubes are more or less sensitive to light. The counting tube should preferably be grounded to avoid any possible spurious counts. Daily background counting is performed and corrected for. On measuring high activities, minor variations in background count need not be considered.

The specimens from each investigation are invariably compared with similarly prepared P^{32} -standard several times daily, in order to correct for decay rate. The registered impulses from a specimen are approx. normally distributed and the mean error equalling $\pm \sqrt{n_1}$, where n_1 , is the number of impulses. In general about 4000 impulses are measured from each specimen in the present investigation.

Description of the method.

The method here applied has been propounded by HEVESY and ZERAHN and, later, in various respects, modified by NYLIN. In his experiments, NYLIN has obtained, almost, identical values from dried and ashed drawn samples and prefers the former method as the more practical. The method can be described as follows:

A radioactive phosphate solution is evaporated in a heparinized sterile test-tube to which blood from the test subject is added. The blood is then transferred into a paraffined shaking bottle that is made to rotate continuously for 2 hours in a water bath at $37^\circ C$. In this way, part of the radioactive phosphate is transferred from the plasma to the blood corpuscles.

Part of the thus activated whole blood is injected with a calibrated syringe into a vein in the arm of the test subject. The needle is then rinsed in situ with a small amount of saline solution. From an arm vein on the opposite side blood samples are drawn at intervals.

From the remaining portion of the active whole blood in the shaking bottle, preparations are immediately made. Their activity is measured later. The hematocrit of the blood in the shaking bottle is estimated from blood drawn into small graduated capillary tubes which have been centrifuged for 30 minutes at 3,500 r.p.m. As a rule, the determinations have been performed on 4 blood samples from the shaking bottle.

The total red cell volume is then calculated from the volume and the activity of injected corpuscles and the average activity of the blood corpuscles taken from the circulation at intervals according to the formula

$$TRCV = \frac{V \times Hct \times I}{D}$$

where $TRCV$ = total-red cell volume in cc.

V = injected blood volume in cc.

Hct = hematocrit from the shaking bottle

I = impulses per gm and per min. of injected corpuscles

D = average impulses per gm and per min. of corpuscles withdrawn from the circulation.

The hematocrit is corrected for trapped plasma.

Preparation of the corpuscular specimens.

Standard samples.

Two samples taken from the shaking bottle are centrifuged and the plasma withdrawn. The corpuscular mass is twice washed with inactive plasma from the subject,

weighed in small glass ampoules, "ashed" with sulphuric acid and nitric acid in Kjeldahl retorts, thus turning all the phosphorus into inorganic phosphate. Heating and dilution with distilled water are performed and to part of the resulting solution are added excess inactive sodium phosphate and magnesium ammonium. A precipitate of magnesium ammonium phosphate is thus obtained (duplicate samples from each Kjeldahl retort). The precipitates are suction-filtered through a perforated aluminium dish covered by a thin sheet of filter paper. Whilst being washed with dilute ammonia, the precipitate is levelled by the use of a glass rod.

The dilution is performed in order to disclose impulses of the same order of magnitude as the samples with-drawn from the patient. This is an advantage from the point of view of the technique of measuring.

Samples drawn from the patient.

The drawn samples are centrifuged and the plasma removed. The corpuscular mass is weighed before and after drying in a thermostat at a constant temperature of about 80° C. A constant amount of this dried corpuscular powder is weighed in aluminium dishes.

Commentaries to the method.

1. Comparison between the different ways of preparation.

As a link in the calculation of total red cell volume a quotient is set down between the number of impulses per minute and gramme for injected and drawn corpuscular samples. Since the values of the dividend and divisor are based on two different modes of preparation, it is indispensable to compare them.

In the ashing method several operations are involved. The results obtained were correspondingly found less reproducible than those obtained by the drying method, which only involves drying and weighing.

By taking 10 samples from each of 8 and 13 individuals respectively, an error of 3.9 per cent was observed for the first method and 1.9 per cent for the latter. The drying technique, however, is not reliable in the case of strongly active standard samples, since it is necessary to dilute them. It is inconvenient to carry out such a dilution with a large amount of powdered unlabelled corpuscles.

A certain numerical difference is noted when comparing values obtained from simultaneous samples from one and the same individual by the two methods. In a *t*-analysis this difference will not be found to be significant ($d = 2.2 \pm 1.7\%$) and from the investigations carried out it is difficult to decide whether or not it constitutes any real difference of practical importance.

Supposing that such a systematic difference occurs, it would be extremely useful if its value could be determined. This, however, presupposes a comprehensive investigation, seeing that the same actual difference cannot be presumed to occur at low, as at high, impulses. It would be misleading to attempt to compute, from the present limited material, a formula for such a difference, and to apply it for practical use.

When obtaining the quotient ashed injected samples/dried drawn samples, the possibility of a systematic error cannot be entirely disregarded. In investigations to compare different groups of individuals, the same results, however, are arrived at, whether correction for a systematic error has been made or not.

Consequently, there is justification for applying the quotient ashed injected samples/dried drawn samples and to use this quotient here in preference to that of ashed_i/ashed samples considering that the latter involves the greater error. In other words, when using the latter quotient, more numerous investigations or less reliable information have to be taken into account.

2. *Regarding the washing of the active blood corpuscles.*

In the general description of the method it was stated that the labelled blood corpuscles were washed twice before the preparation for measurement in the described manner. The question whether any plasma activity adheres and, if so, to what extent, after these washings should be inquired into.

In 4 tests, repeated washings took place (5—6). During this the activity of the washings decreased until the third and then increased again. This gradual increase originates possibly, either in some slowly progressing injury, caused by the forementioned manipulations to the blood corpuscles, or in an accumulation of active phosphate at the corpuscular surface that is finally washed away. It is, on the other hand, less likely that adhering plasma causes the increase. Considering that the result of the third washing has been that only about 0.3 per cent of the activity of the blood corpuscles has been absorbed by the washing solution, not more than 2 washings have seemed justified. The slight advantages of a third washing have not been thought to outbalance the risk of injuries to the blood corpuscles.

There is, accordingly, a certain, though slight, plasma activity adherent to the standard samples. But in view of the fact that the comparisons are made between these samples and the drawn samples of blood corpuscles, a small adherence of plasma activity of the same order of size can be tolerated in the case of the latter.

In 10 tests, the activity of the adherent plasma in the drawn samples has been estimated at about 0.4 per cent before any washings of the blood corpuscles have taken place. In other words, a washing of the drawn blood corpuscles can hardly be considered to be justified.

3. *The activation procedure.*

By shaking the blood sample with a certain amount of evaporated active phosphate solution of negligible weight, part of it will pass into the blood corpuscles while the remainder will dissolve in the plasma.

A high concentration of activity (impulses/g and min.) will be found in the plasma at the beginning of the activation procedure. This diminishes successively, while the activity of the red blood corpuscles increases. Thus, the distribution quotient of P^{32} between the blood corpuscles and the plasma gradually increases.

The trend of this increase may be influenced by the hematocrit figure.

A high hematocrit can be expected to lead to a more rapid intrusion of P^{32} into the corpuscle phase. The result of a high hematocrit should, accordingly, involve an increase in the quotient between the activity concentrations, above all when the reduction in the activity concentration of plasma is rapid. On the other hand, at a low hematocrit the quotient increases principally owing to the rapid increase in the activity concentration in the blood corpuscles.

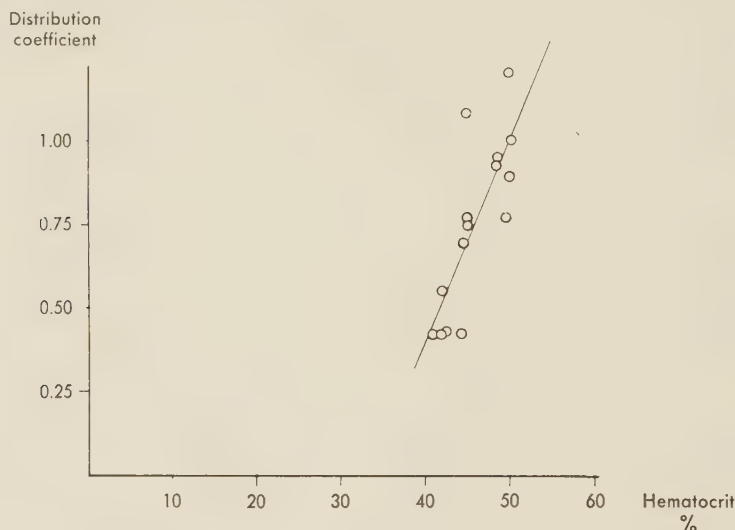


Fig. 1. Relationship between hematocrit-figure and distribution-coefficient in vitro after 2 hours incubation with radio-active phosphorus (males).

Calculating the distribution coefficient which denotes the ratio of the radioactive phosphate content of corpuscles and plasma of the same weight after 2 hours' activation for hematocrit values between 35–50%, we found the distribution coefficient to increase with increasing hematocrit figures ($r = 0.80 \pm 0.09$, $n = 15$ men), fig. 1.

However, probably an increased hematocrit cannot generally be concluded to involve an increased distribution coefficient. A lower distribution coefficient at a very high hematocrit than at a moderately high hematocrit will not gainsay the above arguments.

In 16 cases, the hematocrits have been determined before and after shaking. No significant differences have been noted ($d = 0.27 \pm 0.21$). Calculating the error of method in the hematocrit determinations, we arrive at a figure of 1.9 per cent. The same error is observed when the hematocrit of individuals is repeatedly determined (see page 47). In 4 cases, the *MCD* of the blood corpuscles has been determined before and after shaking, and no significant difference was observed.

This and other observations indicate that the blood does not actually undergo any marked change as regards the relation blood corpuscles/plasma during the shaking procedure.

4. Plasma injections.

The injection of active plasma in a subject produces a labelling of the blood corpuscles. In the survey of the literature, an account was given of such investigations according to which the uptake by the blood corpuscles of radioactive phosphate can be described by means of an exponential curve. It should be borne in mind that the results of the activation of corpuscles *in vivo* and *in vitro* are not entirely comparable. This applies in particular to the mixing in the blood stream and to the loss of plasma activity, for instance, to the tissues.

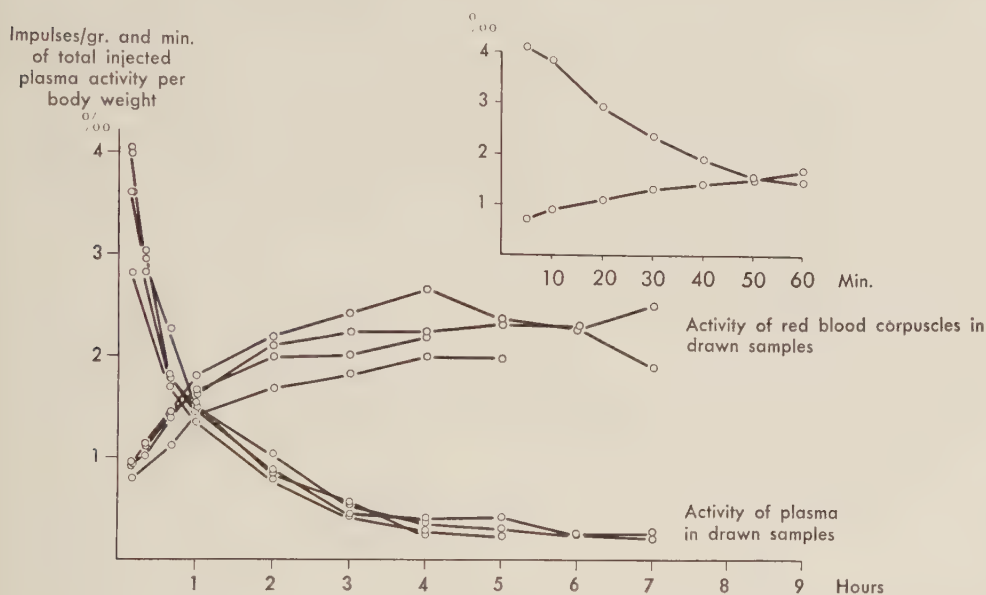


Fig. 2. Activity of red blood corpuscles and plasma after injection of labelled plasma. In the right upper corner of the figure, the means of the corpuscular and plasma activities from the four cases during the first hour are to be found. The uptake by the corpuscles of activity from the injected labelled plasma is calculated as follows:

5 min.	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
2.3 %	2.9 %	3.5 %	4.2 %	4.6 %	4.9 %	5.4 %

The four cases obtain, on an average, a hematocrit of 0.44 and a distribution-coefficient of 0.60. (See further the text, page 43).

Fig. 2 illustrates 4 cases in which the activity of blood corpuscles and plasma (impulses/g and min.) from drawn samples is expressed in per mille of injected total plasma activity per kilogram of body weight. The plasma activity is seen to decrease while that of the blood corpuscles increases. Five to six hours after the injection of the plasma the increase in the corpuscular activity is terminated and then a successive decrease with time takes place. The plasma activity decreases slowly during the same time.

The 4 cases whose curves are reproduced here show an average hematocrit of 0.44 and a distribution coefficient of 0.6. As previously stated it is of importance to determine

the hematocrit, as the relation between hematocrit and distribution coefficient has to be taken into account. Since no determinations of the total red cell volume have been made in these cases, an average value has been inserted which was calculated from the regression line between total red cell volume and body weight, as seen below.

From the thus calculated total red cell volume and from impulses per minute and per gramme of the corpuscles in the drawn samples, the percentage uptake by the blood corpuscles of the injected plasma activity can be estimated for the various times. These percentages are stated in *fig. 2*.

Plasma curves observed when whole blood was injected correspond to similar curves obtained after injecting plasma only. However, it should be noted that the former values during the observation period of 2–60 minutes throughout exceed the latter values to a small extent.

This might be attributed to chance, for there is a marked correlation between the values in each individual. On the other hand, such a difference is to be expected as some activity passes during this experiment from the corpuscles to the plasma.

5. Injection of washed corpuscles.

Injections of washed active blood corpuscles suspended in inactive plasma in the hematocrit ratio have been performed in normal subjects. The corpuscular activity of the drawn samples has been observed up to one hour after the injection. In this connection, a group of 4 cases has been studied with an average hematocrit of 0.42 and an average dispersion coefficient of 0.6.

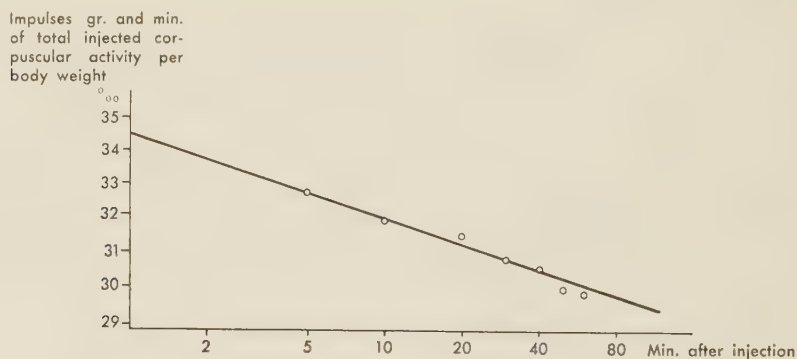


Fig. 3. Changes in corpuscular activity after injection of labelled blood corpuscles.

(Means of corpuscular activity of four cases plotted on double logarithmic scale).

By means of extrapolation the loss of activity from the labelled corpuscles is calculated as follows:

5 min.	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
5.1 %	7.4 %	8.8 %	10.6 %	11.5 %	13.1 %	13.4 %

The four cases obtain, on an average, a hematocrit of 0.44 and a distribution-coefficient of 0.60. (See further the text, page 44).

The corpuscular activity is found to fall slowly during an observed period of 5–60 minutes (i.e. after the termination of the mixing phase). Linear extrapolation on a double

logarithmic scale took place to the abscissa O (i.e. in absolute figures, 1 minute), the straight line representing, comparatively well, the observed values. Extrapolation might of course have been made to another value. However, no other value has been found that would have been more suitable.

In this way, it is possible to draw a curve where time is an independent variable (x) and corpuscular activity a dependent variable (y). *Fig. 3*. The activity of the blood corpuscles in the drawn samples is expressed in per mille of injected total activity per kilogram of body weight. It emerges from *fig. 3* that the activity of the blood corpuscles gradually decreases. Also the percentage decrease of the corpuscular activity from the extrapolated zero value has been denoted.

6. Uptake and loss of activity by the corpuscles in injections of labelled whole blood.

In the present work, the method for the determination of the amount of blood corpuscles has been worked out from injection of labelled whole blood. The mean of the activity per gramme och per minute of the corpuscles from drawn samples have formed a basis for the determinations of the total cell volume. It should, accordingly, be worth while to inquire further into the activity changes in blood corpuscles and plasma at injections of whole blood.

It must be borne in mind that only the activity of the injected plasma is rapidly diluted in the body. The radioactive phosphorus contained in the injected blood corpuscles is not diluted, these corpuscles, however, are mixed with the uncharged blood corpuscles of the patient.

The dilution of the plasma activity is very rapid. The plasma activity falls to such an extent that in practice any further activation of the injected labelled blood corpuscles by the plasma will not occur. Nevertheless, the plasma activity, though low, will still label the patient's own inactive blood corpuscles.

The activity changes *in vivo* could, accordingly, be divided into two components that have already been separately studied, viz., on the one hand, a labelling of the uncharged blood corpuscles from the plasma and, on the other, a loss of activity in the plasma from the injected high-active blood corpuscles.

In spite of the fact that this latter process is quite considerable, it will only slightly increase the plasma concentration in view of the large plasma volume of the patient. It should, therefore hardly contribute to the first-mentioned process, i.e. the labelling of the inactive blood corpuscles of the patient from the injected plasma. This conclusion is also corroborated by the results obtained in experiments in which washed active blood corpuscles have been injected, and the plasma activity studied. A very low plasma activity was found.

In view of these arguments, a curve for injected whole blood could be obtained by addition on the basis of the above discussed curves for injected active blood corpuscles and active plasma only.

The benefits of the results from the forementioned tests with injections of blood corpuscles and plasma only, with an approximate distribution coefficient of 0.6, will

now be apparent. Labelled whole blood, showing a distribution coefficient of 0.6 as the forementioned cases, was injected in 3 patients, and total activity of the corpuscles and plasma determined. These values, and the above recorded percentage values for loss and uptake of activity, have then been interlinked, supplying values plotted in *fig. 4*.

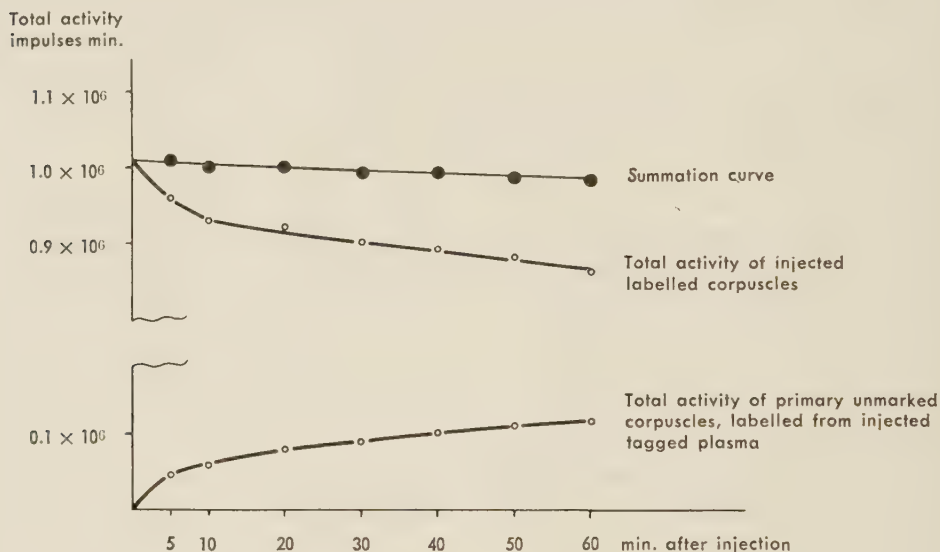


Fig. 4. Changes in total activity after injection of labelled whole blood.

In the *fig. 4* are seen the amounts of P^{32} given off by the active blood corpuscles and taken up by the inactive ones from the plasma. The resulting values plotted also at the top in *fig. 4* is an almost straight and horizontal line. This lends support to the hypothesis that loss and uptake of P^{32} by the corpuscles balance each other, for if the a priori summation curve be straight and horizontal, it follows that

- 1) the extrapolation at injection of blood corpuscles only, is satisfactory,
- 2) the addition procedure corresponds to reality,
- 3) no mathematical interferences have taken place, causing systematic errors.

It should be added that the appearance of the noted summation curve suggests that the results from the three test groups that form its basis are reliable.

The trend of the summation curve indicates that the total activity of the circulating corpuscles is not notably changed in the course of a time interval of 0–60 minutes. There is, therefore, justification for calculating the total red cell volume from the ratio of the values of the total activity of injected blood corpuscles and those of the activities of blood corpuscles recorded at different times after injection of whole labelled blood. Between the activities from drawn corpuscles should only accidental variation exist.

Extrapolation is not required when injections of labelled whole blood are applied.

In 11 cases, the activity of the blood corpuscles from drawn samples has been deter-

mined at intervals between 5 and 60 minutes after injections of whole blood. On one of these occasions ten separate samples were drawn and the activity of each determined. This was done to estimate the error in an individual observation. When comparing the variation between the observations, made at different times, with the recorded error of the method, no trend can be found. This was the case for each of the above mentioned determinations in spite of the fact that the distribution coefficient varied between 0.6—1.5. There is justification for stating that the forementioned tests speak in favour of the whole blood method. Should any errors have occurred in the above reasonings, they should have been traceable in the recorded values from injections of whole blood by tending divergences in the values obtained at different times.

In 4 investigations, *in vitro*, the total red cell volume has been determined by the P^{32} -method on previously known cell volumes. In these experiments about 2 ml. of labelled whole blood previously incubated for 2 hours at $37^{\circ} C$ added to about 300 ml. inactive blood. No significant difference could be found between the cell volume calculated from radioactive data and that obtained from volume and hematocrit figure of the blood.

7. The error in the determination of the total red cell volume.

In order to arrive at the total red cell volume, activity determinations, as described on pages 39, 40, have to be carried out and the hematocrit from the shaken bottle also determined. The total red cell volume is calculated from the activity and volume of injected blood corpuscles and the corpuscular activity of drawn samples.

The error in the standard sample, performed with the ashing method, was found to be 3.9%, and in the drawn sample, carried out by the drying method, 1.9%.

In determining the total red cell volume activities of several standard samples (two precipitates from each of two ashed specimens) and of several samples drawn at intervals from the circulation are measured. The error in the values has amounted to 1.7% and 1.2% respectively.

The error of the method inherent in the hematocrit procedure applied here amounts to 2.1%. The result is based on a large number of hematocrit determinations from several individuals. In our experiments in estimating the total red cell volume the hematocrit is determined 4 times, thus the error is found to be 1.0%.

We have furthermore to consider the error in the volume of the blood, injected by a syringe. It was found to be 0.2%.

After having calculated the error in the divisor and the 3 factors in the dividend, an estimation of the error in the quotient can be made. It is, approximately, a little greater than $\sqrt{(1.0)^2 + (1.7)^2 + (1.2)^2 + (0.2)^2} = 2.3\%$.

From a statistical point of view this lastmentioned calculation is correct, as can be seen from Kendall, part I, Section 367, ex. 14.15.

Thus the error that is on an average inherent in the observed values of total red cell volume, is estimated at 2.3%.

Because of the injection technique, traces of blood remain behind when emptying the syringe, which constitutes a further error. This error can be disregarded, as adhering corpuscles were found to make out only 0.3% of the injected cell volume.

When viewed against the background of the accidental errors inherent in any determinations of the total red cell volume, and compared to the distribution in the values of the total red cell volume in the control cases, the forementioned error looks inconsiderable. This is evident also from the fact that 0.3%, in absolute figures, is less than the class interval applied in estimates of red cell volume.

8. "*Trapped plasma*".

At determinations of the hematocrit, the blood corpuscles should be packed so as to answer to Koeppe's criterion by means of an appropriate centrifugation work. Nevertheless, a certain amount of plasma is trapped among the cells.

Several methods to determine this factor have been tried. At direct methods, the plasma has been carefully withdrawn and the erythrocytes suspended in a solution, to which an indicator is added. At indirect methods, on the other hand, the separated plasma, containing the indicator, is compared to a plasma standard, to which a definite amount of the indicator is added. MC LAIN & RUHE (1949), however, arrived at the conclusion that the indirect methods are unsuitable owing to the varying results that could be obtained.

By using the direct method, the trapped plasma was calculated at 3 per cent of the packed red cell column (HAHN & HEVESY, 1942; MAIZELS, 1945), at 4 per cent (GREGERSEN & SHIRO 1938) and at 4.5 per cent (SHOHL & HUNTER, 1941).

On the basis of these investigations correction factors for the hematocrit value for trapped plasma have been introduced. Generally, as in the present investigation, the correction factor of 0.96 has been applied.

EVANS BLUE METHOD

All dye solutions used in the present investigation came from the same lot of Evans blue, as delivered by Messrs. Eastman Kodak Co., Rochester, N.Y. Sterile solutions with physiologic salt in a concentration of 3 mg T-1824/ml have been prepared and ampullated, 5 ml per ampulla. No precipitation has been noticed in these ampullae after 2 years.

The readings have been made in Beckman Du spectrophotometer at an absorption maximum for Evans blue plasma at 620 m μ . Double readings have been performed for each sample, and oxyhemoglobin controlled by readings at its absorption maximum at 540 m μ .

The readings have been made against a blank preparation, viz, a plasma sample drawn before the injection of Evans blue.

In the course of the investigations, repeated standardizations of a preparation have been made. With regard to the different concentrations of the dye, the extinction values

follow Low-Beer's law, represented a straight line by means of which the extrapolated extinction values have been transmitted to concentration values.

Thanks to the two readings, viz, at 620 m μ and 540 m μ in each separate case, it has been possible to form a conception of the existing degree of hemolysis. Mild degrees of hemolysis do not affect the result of a reading at 620 m μ . When more marked, i.e. observable to the naked eye, the sample has been excluded. Accordingly, there has been no reason to apply GIBSON and EVANS' formula (1937) for correction for the degree of hemolysis. The reliability of this formula has indeed been questioned by, among others, PHILLIPS (1943) and NOBLE and GREGERSEN (1946). GREGERSEN (1951) states that these corrections for hemolysis are laborious and unsatisfactory.

The blood has been prevented from coagulation by being transferred into heparinized test tubes. After centrifugation the plasma has been withdrawn. Consequently, there has been no need to calculate with any dilution effect.

The injection of Evans blue has taken place immediately after that of labelled blood corpuscles, with a similar syringe. By repeated aspirations the syringe is rinsed off from dye.

Mixing and disappearance.

It is important to ascertain, if possible, how long the mixing phase approximately lasts, seeing that, during that period, no representative samples are obtainable. Samples, drawn from different major blood vessels, reveal on each occasion different values. Since samples drawn immediately after an actual mixing, are, undoubtedly, the most useful, because they provide the best information of disappearance, it is not advisable to a priori calculate with too prolonged mixing phases. It is necessary to attempt an estimation of the time at which disappearance sets in. If this point is supposed to coincide with that of the completion of the mixing phase, samples from that time will suffice, but, on the other hand, if it begins during the mixing phase samples must be taken at intervals after the end of the mixing phase. By extrapolation to the time when disappearance begins, by means of any appropriate statistic-mathematic method, an estimate of the true value is obtained. If, disappearance is presumed to set in already at zero, extrapolation must take place up to the y axis.

To conclude from results that will later be reported, the mixing phase of Evans blue in the circulating blood seems completed after 15 minutes in cardiac insufficiency, in so far as the major vascular regions are concerned. When there has been reason to suspect that disappearance has set in already at zero, extrapolation has been performed to the y axis from the values obtained between 15 and 60 minutes. In the present investigation, semilogarithmic extrapolation has been preferred, in view of the fact that, the different values from the stated times will then best fit in with a straight line.

When disappearance has varied markedly from one individual to another, it is evident that the Evans blue method presuppose repeated sampling for a fairly prolonged period to render the extrapolation reasonably reliable.

METHODS FOR INVESTIGATION OF BONE MARROW

Smears of bone marrow, obtained by sternal puncture, have been prepared. They have been stained ad modum May-Grünwald and Giemsa ($pH = 6,8$).

Apart from a qualitative estimation, the bone marrow smears have been subjected to differential counts of 2,000 nucleated cells,*) a more thorough differentiation being made for cells belonging to the erythropoiesis. In addition, the mitotic figure in the various erythroblastic stages has been stated.

In addition, further, to this differentiation of the erythropoietic cellular elements, with regard to nuclear structure and stainability, a division has been performed for every kind of cell into macroforms and "normal" forms, according to diametric size, as follows from *table 3* (see Hämatologische Tafeln Sandoz).

Table 3.

Proerythroblasts	Basophilic erythroblasts	Polychromatic erythroblasts	Orthochromatic erythroblasts
Normal —17.5 μ	—15.5 μ	—12 μ	—10 μ
Macro 18 μ —	16 μ —	12.5 μ —	10.5 μ —

The size of the diameters has been determined by ocular micrometry.

This procedure, viz, to divide a certain type of erythroblast into two classes according to size, is applicable when no particularly exact value for the *MCD* is required, a relative value being sufficient.

For the purpose of checking the applicability of this method, the *MCD* of polychromatic erythroblasts has, in a number of cases been calculated according to both the simplified and the more exact method, a highly significant correlation being obtained ($r = 0.73 \pm 0.13$, $n = 13$; $t = 5.6^{***}$).

METHOD FOR MEASURING DIAMETER OF RED BLOOD CORPUSCLES
(*MCD*)

The method used in the present investigations is identical with that described in the investigations made by GOLDBERG, GUNVALL, HAMMARSTEN and LINDGREN (1948). Here, a summary will be given of this method, which is principally ocular micrometry of dry preparations in oil immersion.

A thin smear of one drop of blood is immediately dried, by waving in the air, then fixed in methanol, stained in May-Grünwald and Giemsa's stain, rinsed in water and buffered with phosphate (Sørensen; $pH = 6.8$).

When measuring, the ocular is arranged so as to make the 10 scale gradations on the eye-piece represent 10 μ on an object-micrometer. As an objective, an ordinary oil immersion lens is used.

*) performed by miss Thilde Springenschmid, the Clinical Central Laboratory, Södersjukhuset.

In the present investigation, 100 or 200 red cells are measured, each in one diameter only parallel to the longitudinal axis of the slide. Frequency curves of the cells with a class interval of 0.5μ are drawn.

Since, accordingly, the measurements in the present investigations and those of the forementioned authors have been performed in an identical way and, in fact, by the same person*) values for normal cases and error of method will be taken, from the work of those authors. They arrived at an average method error of $0.09 \pm 0.012 \mu$. They could not find any differences in the means in simultaneous determinations of red cell diameters by "wet" and "dry" technique, respectively, (both being performed in oil immersion).

The mean red cell diameters in healthy subjects have been tabulated in *table 4* acc. GOLDBERG et al. (1948).

Table 4. Mean Red Cell Diameter in Healthy Subjects acc. to
Goldberg, Gunvall, Hammarsten & Lindgren.

	Number of cases	Mean diameter ($\bar{x} \pm s\bar{x}$) μ	Standard deviation (s) μ	Coefficient of variation %
Males	33	7.67 ± 0.02	± 0.14	1.9
Females	57	7.69 ± 0.02	± 0.13	1.7

From the table will be seen that no significant difference has been found in the means between males and females.

OTHER INVESTIGATION METHODS

Determinations of osmotic resistance of the erythrocytes.

The osmotic resistance of the blood corpuscles has been determined by dilution with *NaCl* solutions in concentrations rising from 0.20 per cent to 0.60 per cent with an 0.02 per cent difference in concentration between the solutions. The concentration is stated for solutions in which an initial and total hemolysis has proved ascertainable. Total hemolysis (i.e. maximal resistance) has been defined as a condition in which after centrifugation, no sediment of erythrocytes has been observable at the bottom of the tube. Initial hemolysis (i.e. minimal resistance) has been regarded as manifesting itself by a faintly red tint in the fluid above the bottom layer of erythrocytes.

Determination of serum iron.

Determinations of serum iron have been made according to the method suggested by AGNER. It is based on earlier experiences by HEILMEYER and PLÖTNER (1937), BARKAN and WALKER (1940) and VAHLQUIST (1941). At Södersjukhuset, this method has given a standard error in single determinations of $\pm 3.2 \gamma$ (BIÖRCK, 1948).

*) Febe Gunvall

Liver tests.

In blood serum, bilirubin has been determined according to JENDRASSIK and GROF (1938), alkaline phosphatase according to BUCH and BUCH (1939), citric acid according to PUCHER, SHERMAN and VICKERY (1936) and Takata's reaction according to HAFSTRÖM (1935).

In addition, determinations of total protein in the serum as well as of fractionized protein, have been carried out according to the biuret method (KINGSLEY 1939); checked by the KJELLD AHL method.

Hematic values.

The hemoglobin (*Hb* %) has been photometrically determined as acid hematin. The standardization has been performed in such a way as to make *Hb* 100% correspond to 15 *GmHb*, acc. VAN SLYKE, or to a standard with known amount of cyanhemoglobin.

Table 5. Hemoglobin and red cell count in various blood samples in decompensated and compensated heart failure.

Comparisons			Mean difference
Decompensated	<i>Hb</i>	{ finger-venous blood	-3.6 ± 1.0
			$3.6^{**} (20)$
		{ arterial-venous blood	0.3 ± 0.9
			$0.4 (12)$
	<i>RBC</i>	{ finger-venous blood	-0.18 ± 0.06
			$3.0^{**} (19)$
		{ arterial-venous blood	0.02 ± 0.04
			$0.5 (11)$
Compensated	<i>Hb</i>	{ finger-venous blood	$+1.3 \pm 2.6$
			$0.5 (9)$
		{ arterial-venous blood	-0.6 ± 1.2
			$0.5 (9)$
	<i>RBC</i>	{ finger-venous blood	-0.17 ± 0.15
			$1.1 (7)$
		{ arterial-venous blood	-0.10 ± 0.07
			$1.4 (6)$

The values are $\frac{d \pm \varepsilon_d}{t (n)} = \text{difference} \pm \text{error of difference}$

$$t = \frac{d}{\varepsilon_d} (\text{number of cases})$$

In each test, two determinations have been made, the error amounting to 1.2% (HAMMARSTEN, et al., 1950).

Erythrocyte counts (*RBC*) have been made in HAYEM-JORGENSEN'S solution, i.e. a dilution of 1:200 in controlled pipettes and counting chambers. The error in a single determination is 0.25 mill/mm³ (HAMMARSTEN, et al., 1950).

The reticulocyte counts were made in dry preparations.

Hb and *RBC* were determined from finger blood, venous blood and, in a few instances, from arterial blood. (Table 5).

In the decompensated cases, significant lower values of *Hb* and *RBC* were noted in the finger samples as compared to the venous ones. On the other hand, in a comparison between arterial and venous blood no significant differences were ascertainable in these samples.

In the compensated cases, no significant differences were observed between these samples of finger, venous and arterial blood.

The lower values obtained from finger blood in decompensated cases are, no doubt, due to the fact that tissue fluid is included in the blood at the sampling in the occluded finger.

Values of *Hb* and *RBC* from finger and venous blood are therefore not quite comparable in decompensated cases. Still, if the values from finger blood are multiplied by a constant 1.04, obtained from the percentage mean difference between these samplings, the finger blood values should become more comparable with the venous ones.

When the observed and estimated venous hematic values are computed, a material is obtained the mean of which slightly deviates from that obtained with observed venous blood values only. (The correction constant is merely an estimated value).

Blood gas analyses.

Oxygen content and oxygen capacity of the arterial blood have been determined, always in duplicate, on the van Slyke apparatus using the technique of VAN SLYKE and O'NEILL (1924). Correction has been made for physically dissolved oxygen. From these values the oxygen saturation has been derived. In addition the *CO*₂-content of the arterial blood has been determined.

STATISTICAL METHODS

In the analysis of primary data, each sample has, as a rule, been subjected to calculations of mean ($\bar{x}$), standard deviation (s), standard error of mean ($\epsilon_{\bar{x}}$) and, in some instances, range (w).

It has often been necessary to resort to more complicated statistical methods of analysis. Thus, regression and correlation analyses have been applied, in order to ascertain the occurrence of relationships, as well as *t*-analysis and analyses of variance, in comparisons of statistics. No detailed account of the statistical methods of analysis will be given here, since such descriptions are available in any statistical text-book.

Because of the disagreement that prevails regarding the levels of significance, it should be mentioned that, in the present work, the following intervals of significance have been used (CRAMÉR, 1946):

	$p > 0.05$	not significant	
$0.05 \geq p > 0.01$		almost significant, denoted by	*)
$0.01 \geq p > 0.001$		„ „	**)
$0.001 \geq p$	highly	„ „ „	***)

The distribution in the populations from which samples have been drawn have not throughout been definable as approximately normal. For that reason, recourse has in certain instances been had to transformations of variables. This has been done, as soon as experiences from earlier investigations have indicated such a procedure, or when, according to a priori knowledge, a limit has to be taken into account, approached but not exceeded by the observed values.

Throughout, the logarithmic scale has been preferred, when transformations have been regarded as expedient.

ADDENDUM:

Measurements of the absolute activity of the standard specimens have been performed at Chalmers Tekniska Högskola, Göteborg (Ass. Prof. K. Ziemen). On account of these measurements the total activity of the injected labelled whole blood has been calculated to amount to $20 \mu C$, on an average.

CHAPTER VI

Material

COMPOSITION OF CONTROL MATERIAL

In the survey of the literature, various "normal values" have been given for the total red cell volume by the P^{32} method, including values from investigations by NYLIN & HEDLUND. In order to secure a basis for comparison with the pathologic material examined by the author, control cases were taken from the latter investigations.

The author has obtained a further number of control cases, simultaneously with the collection of the material of cardiac insufficiency. The control cases comprise men and women. The male and female control materials, are differently composed with regard to the clinical condition. In this respect, the cases can be divided into three groups, viz, one comprising cases that may be regarded as healthy, i.e. policemen, students and laboratory assistants, and a second group including cases previously admitted to medical

Table 6. Survey of control cases.

Males	Volume of erythrocytes cc/m ²	Age years	Body surface m ²	Hematocrit %
Healthy subjects	1132 ± 38 137 (13)	ca 29 18—40	1.96 ± 0.04 0.13 (13)	44.0 ± 0.9 3.3 (13)
Neurasthenic subjects	1136 ± 37 139 (14)	32 19—44	1.90 ± 0.04 0.14 (14)	44.6 ± 0.9 3.4 (14)
Convalescents	1169 ± 47 132 (8)	30 21—43	1.86 ± 0.08 0.21 (8)	43.1 ± 0.9 2.4 (8)

Females	Volume of erythrocytes cc/m ²	Age years	Body surface m ²	Hematocrit %
Healthy subjects	847 ± 45 111 (6)	26 21—32	1.70 ± 0.06 0.15 (6)	38.9 ± 0.6 1.5 (6)
Convalescents	1035 (1)	29	1.53	42.0

$\bar{x} \pm \varepsilon_{\bar{x}}$ = mean ± error of mean
 The values are s (n) = standard deviation (number of cases)
 and $\bar{x}$ = mean
 and w = range

or psychiatric departments for headaches or general symptoms of neurasthenia. This group also comprises a case of acute, though mild, lumbago in which the total red cell volume was determined soon after the onset of the complaint. The third group, comprises cases of gastric ulcer, gastritis, infections in the upper air passages, as well as one case of pleuripneumonia. Common to these latter cases is that the total red cell volume has not been determined until just before their discharge as healthy.

Common features to the control materials both of men and women are that they all show normal cardiologic and hematologic conditions, and that all are in a good nutritive condition. All, including also the group of "healthy" cases, were physically examined. In several cases, including those admitted for observation of symptoms of neurasthenia, as well as healthy and convalescents, amounting to half the material, the heart volume has been determined according to LYSHOLM, NYLIN and ZACHRISSON in JONSELL'S modification. Further, electrocardiograms and venous pressure have been taken in cases with symptoms of neurasthenia, when indicated.

Certain data from the different groups of the control materials are to be found in *table 6*. The findings in all the examinations, including age, body surface, hematocrit and total red cell volume per m² of body weight, agreed in all the groups. The differences in means seem inconsiderable when compared with the errors of the means.

From this, it follows, that these cases can form the basis of a probably satisfactory control.

Total red cell volume in the control material.

The red cell volume, total and expressed per m² of body surface and kg of body weight, will be seen from *table 7*. It emerges that the total red cell volume is bigger for men than for women ($t = 5.37^{***}$) The difference is highly significant.

Table 7. Total volume of erythrocytes in control cases.

	Total amount of erythrocytes cc.	Amount of erythrocytes/ body surface cc/m ²	Amount of erythrocytes/ body weight cc/kg.
Males	2166 ± 56 331 (35)	1131 ± 22 131 (35)	29.6 ± 0,7 3.9 (35)
Females	1460 ± 103 274 (7)	868 ± 44 115 (7)	24.0 ± 1,1 3.0 (7)

The values are $\bar{x} \pm \frac{\varepsilon_{\bar{x}}}{s} = \text{mean} \pm \frac{\text{error of mean}}{\text{standard deviation (number of cases)}}$

Table 8. Some correlations of total red cell volume in male control group.

Body surface	Body weight	Heart volume
$r = 0.62 \pm 0.10$ (35)	$r = 0.56 \pm 0.12$ (35)	$r = 0.67 \pm 0.13$ (17)

The values are $r \pm \varepsilon_r = \text{coeff. of correlation} \pm \text{error of coeff.}$
(n) (number of cases)

As far as the men are concerned, the total red cell volume is correlated to heart volume, body surface and body weight. The correlation coefficients are to be found in *table 8*. From this, it will be seen that the best correlation applies to heart volume and body surface (see *fig. 5*).

Correlation to body surface is preferable to that of body weight, seeing that the body weight in one and the same individual is subject to fairly considerable variations in the content of water and fat. The latter factor is particularly notable when a pathologic material is concerned.

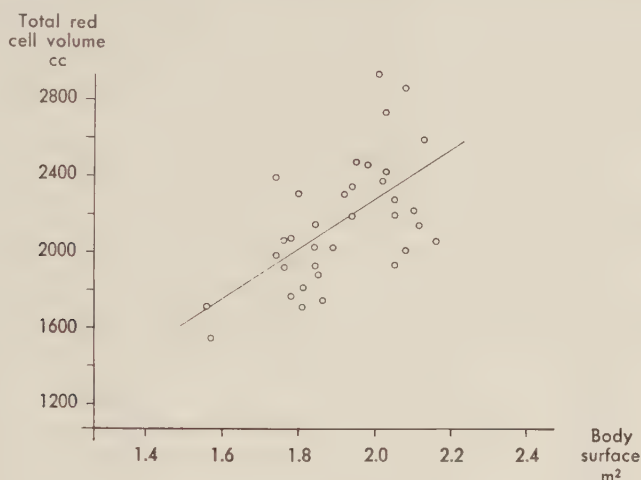


Fig. 5. Relationship between body surface and total red cell volume in normal males.

Within the range 18–44 years applied in this instance to men, no relationship could be observed between total red cell volume and age. Nor has any correlation been found between total red cell volume hematocrit and *Hb* (males) respectively.

The total red cell volume in men agrees with the values obtained with radioactive iron by GIBSON and others (1946), with P^{32} by BERLIN and others (1950) as well as with those of STERLING and GRAY (1950) with Cr^{51} stated for normal males (*Table 9*).

With regard to females, BERLIN and others (1951) noticed, like the present author, a lower total red cell volume than for males.

COMPOSITION OF MATERIAL OF CARDIAC INSUFFICIENCIES

The material of cardiac insufficiencies that is submitted in this investigation consists of cases all of which have displayed symptoms of manifest insufficiency. They have all been in need of hospitalization. They have been divided into principal groups of diagnosis and sex, as well as into some subdivisions with regard to the degree of the decompensation.

From a *diagnostic point of view*, the material has been classified into two main groups according to the particular type of heart affection, i.e. whether valvular or vascular. In addition, there is a case of decompensated chronic cor pulmonale.

Table 9. Total red cell volume in normal cases and in convalescents estimated with the aid of radioactive isotopes.

	Number of cases Sex.	Body weight Age.	Total red cell volume, cc cc/kg. cc/m ²	Correction for trapped plasma	Material
<i>Radioactive iron:</i>					
Gibson, J. G., Peacock, W. C., Seligman, A. M. & Sack, T. (1946)	40 males	59-90 kg. 18-24 years	2208 29.7 1150	no corr.	normal subjects
Meneely, G. R., Wells, E. B. & Hahn, P. F. (1947)	28 males		1740	no corr.	normal & convalescents
<i>Radioactive phosphorus:</i>					
Hevesy, G., Köster, K. H., Sörensen, G., Warburg, E. & Zerahn, K. (1944)	21 cases 6 cases	54-72 kg. 47-75 kg.	2107 33.3 1850 30.9	no corr. no corr.	normal subjects normal subjects
Nylin, G. (1945)	19 m. & f.	47-89 kg.	1945 28.9	no corr.	normal & convalescents
Nylin, G. & Hedlund, S. (1947)					
Mayerson, H. S., Lyons, C., Parsons, W. & Nieset, R. T. (1948)	26 m. & f. (6 m. & f.)	41-94 kg. 53-82 kg.	1649 24.9 1896 30.2)	f = 0.915	normal & convalescents normal subjects
Kelly, F. J., Simonsen, D. H. & Elman, R. (1948)	9 cases		2632 33.9 1221		
Reeve, E. B. & Veall, N. (1949)	13 cases		2160 30.4	f = 0.95	normal subjects
Nachman, H. M., Watson, James G., Moore, J. W. & Evans, E. J. (1950)	38 m. & f.	42-91.5 kg. 16-78 years	1850 29.2	no corr.	surgical patients convalescents
Berlin, N. J., Lawrence, J. H. & Gartland, J. (1950)	12 males		29.3	f = 0.96	normal subjects
Berlin, N. J., Hyde, G. M., Parsons, R. J., Lawrence, J. H. & Port, S. (1951)	16 fem.	44-81 kg. 22-48 years	27.0	f = 0.96	normal subjects
Wasserman, L. R., Yoh, T. F. & Rashkoff, I. A. (1951)	26 cases		25.8	f = 0.915	normal & convalescents
<i>Radioactive chromium:</i>					
Sterling, K. & Gray, S. J. (1950)	25 males	64-96 kg.	2351 31.8 1210	no corr.	normal subjects

The first-mentioned group, the valvular, includes patients with mitral defects (mitral stenosis — mitral insufficiency), as well as combined mitral and aortic defects. There is in this group an additional case of mitral insufficiency — tricuspid insufficiency (the diagnosis verified at the postmortem). The forementioned cases represent rheumatic valvular defects.

Cases with cardiosclerotic or hypertensive heart diseases, or a combination of both, have been referred to the vascular group. It has proved difficult to divide this main group into subdivisions with simple diagnoses. Thus, many cases, designated as cardiosclerotic heart diseases have earlier had hypertension, before the insufficiency symptoms had been pronounced, or before the condition had been complicated with a myocardial infarction. Similarly, a physical examination, electrocardiography and roentgen of the heart does not exclude the possibility of a diagnosed hypertension being combined with a cardiosclerotic heart disease. Also, a hypertension may have an arteriosclerotic genesis. Nor has an analysis of different correlations presented later, after the distribution of the material in sub-groups, produced any results diverging from those of an analysis of the whole group. For this reason, it has been treated as a whole.

In some cases in this main group, a lues has been noted, simultaneously with a cardiosclerosis and/or hypertension, without necessarily being the actual cause of the congestive heart failure.

Finally, the genesis of the decompensated case of chronic cor pulmonale was pulmonal sclerosis and mucopurulent tracheobronchitis.

As to the two main groups, the vascular cases can be said to constitute a well-defined, comparatively homogeneous group, while the valvular group is less uniform, since vascular heart affection may, at a higher age, be added to an already existing valvular defect. From the investigations, it is difficult to decide when a vascular component comes in. But even so, the clinical conditions and courses are in these cases, nevertheless, dominated by the valvular heart affections. A possible loss of homogeneity will, from a statistical point of view, only imply that differences between valvular and vascular heart affections may become more difficult to establish.

The *degree of insufficiency* has varied. Synoptically the material has been divided into 3 groups.

Group I comprises patients with severe edemas on the tibias and thighs and slight to moderate edemas in the sacrum and abdominal wall (designated as + + + and + + + +). The degree of edema has been determined when a patient has occupied a half-reclining position in a heart bed and in this way, in the first place, filled his lower extremities with edematous fluid. The degree of edema has been uniformly judged and assessed at the determinations of the total red cell volume. Some patients have had ascites and others slight edemas on the arms. Cases with a venous pressure of over 14 cm. belong to this group.

Group II comprises patients without edemas or with slight to moderate tibial and, possibly, to some insignificant extent, femoral edemas, (designated as + and + +) and with a venous pressure above 14 cm.

Group III includes patients with slight tibial and without femoral edemas, or with no edemas at all, and a venous pressure 12 to 14 cm.

For the most part, the insufficiency symptoms have been of a grave nature in the investigated cases.

Marked edema, considerable dyspnea at slight movements or even at rest, possibly, with attacks of cardiac asthma have been regarded as signs of decompensation in a heart affection. Latent insufficiency symptoms, such as dyspnea at work have, accordingly, not been recognized as signs of decompensation. In an individual case, it may be difficult to ascertain anamnestically when a latent insufficiency is transformed into a manifest one and the decompensation appears at rest. In several instances, the patients have been hospitalized for cardiac symptoms, before the present examination. From the hospital journals it has, *inter alia*, been possible to state whether or not decompensation has been noted at earlier examinations. The application of a continuous digitalis therapy, with, naturally, due regard to the indications for it, has many times afforded a valuable help in determining the time of the appearance of the decompensation. Thus, in most instances, the diagnoses of valvular defects, and hypertension have been established considerably before the occurrence of the decompensation.

In the case of the valvular heart affections, it may be particularly difficult to decide anamnestically, when latent insufficiency symptoms turn into manifest signs. Such cases may for a long time be compensated at rest, in spite of an increasing heart volume and a latent insufficiency. Special attention has been paid to the appearance of edemas on the legs or to data in the hospital journals of any earlier admission for decompensation. Thus, less notice has been given to any restriction of working capacity when compensation has, nevertheless, been present at rest. An additional justification for the application of these principles has been to render comparisons with the vascular group possible. In the latter cases, the decompensation, once it has set in, develops more rapidly. And, in the present investigation, the purpose is, to inquire into the nature of erythropoiesis under decompensation.

Regard to all these factors has, therefore, had to be paid in determining the duration of decompensation up to the present instance.

Immediately after the admission of a patient with symptoms of cardiac insufficiency, the total red cell volume has been determined with the P^{32} -method. At the same time determinations of the plasma volume have, in some instances, been made with Evans' blue, and of the arterial oxygen saturation. Simultaneous tests have been performed with a view to certain hematologic data, viz, *Hb* and *RBC* from venous, arterial and finger blood, number of reticulocytes, serum iron, mean corpuscular diameter and bilirubin, and certain other so-called liver tests. Simultaneously with these tests, other examinations have been made, such as physical examination, measurement of venous pressure, roentgenologic determination of the heart volume according to the method introduced by LILJESTRAND, LYSHOLM, NYLIN and ZACHRISSON in JONSELL's modification. In some instances, also a sternal puncture has been made.

The determinations of the total red cell volume were made on fasting patients, in a recumbent position, as horizontal as the clinical condition has allowed, during the hour required for the purpose.

Then, the patients have been treated for their cardiac insufficiency in the ordinary way with digitalis, preferably cedilanid (HEDLUND 1952), mercurial diuretics, theophylline preparations and, in certain instances, a scarification. The condition has been followed through clinical, and hematologic, examinations. Repeatedly during the treatment, and, at any rate, when the patients become compensated, determinations with the P^{32} -method have been made, in the way previously described, and, at the same time, plasma volume determinations with Evans' blue and determinations of arterial oxygen saturation.

Table 10. Survey of cases of congestive heart failure.

Number of cases examined in at least one of following items.	Vascular heart diseases		Valvular heart diseases		Total number of cases
	males	females	males	females	
	37	10	12	29	88
Total red cell volume (acc. to P^{32} -method)	30	7	8	10	55
Plasma volume (acc. to Evans blue method)	12	1	4	4	21
Mean corp. diam.	35	9	12	28	84
Reticulocytes	18	6	7	9	40
Osmotic resist. of red cells	24	7	8	19	58
Serum iron	22	6	8	11	47
Blood gas analysis	17	4	5	4	30
Sternal puncture	5	3	4	2	14
Liver tests					
Bilirubin	26	4	9	10	49
Phosphatase	10	2	5	12	29
Citric acid	9	2	5	9	25
Takata	22	7	8	19	56
Total protein	21	8	6	18	53

Further in one case of chronic cor pulmonale total red cell volume according to the P^{32} -method and plasma volume with the Evans blue method have been determined. In addition the arterial oxygen saturation has been estimated.

As will be seen from *table 10*, the number of cases varies in the different investigations, as a matter of course, considering that variations in available assistance, etc., must

occur in the case of a work that extends over a long period of time, where the examinations of the patients, accordingly, have been more or less complete. In the individual cases, repeated determinations have been performed during the treatment of a cardiac insufficiency. The total of determinations will, consequently, considerably exceed the number of cases.

In the top line of the table, the total number of examined patients is stated.

The vascular cardiac affections are found somewhat to exceed the valvular in number. In the former group, the males are seen to preponderate, while in the latter group the females are more numerous. The last-mentioned distribution is, as experience shows, typical of a material of cardiac insufficiency.

Table 11. Age in cases of congestive heart failure.

	Males	Females
Vascular heart diseases	62 50—77 (37)	67 61—75 (10)
Valvular heart diseases	48 22—69 (12)	50 29—67 (29)

The values are $\bar{x}$ = mean
 n = range
 (number of cases)

The age distribution will appear from *table 11* where mean and range are stated. The mean age of the cases of valvular heart affections falls below that of the vascular. From a medical point of view, this is quite natural, considering the differences in the genesis of the heart affections. With regard to the vascular affections, a tendency towards a higher mean age is found in women, when admitted for treatment of a cardiac decompensation.

The mean age of the control group is lower than that of the groups of cardiac insufficiency.

CHAPTER VII

Comparisons between the P^{32} and the Evans blue methods

DETERMINATION OF TOTAL RED CELL VOLUME

In a number of cases of cardiac insufficiency, the total red cell volume has been simultaneously determined in decompensation and compensation by the P^{32} -method and the Evans blue venous hematocrit method.

Total red cell volume estimated with Evans blue-method

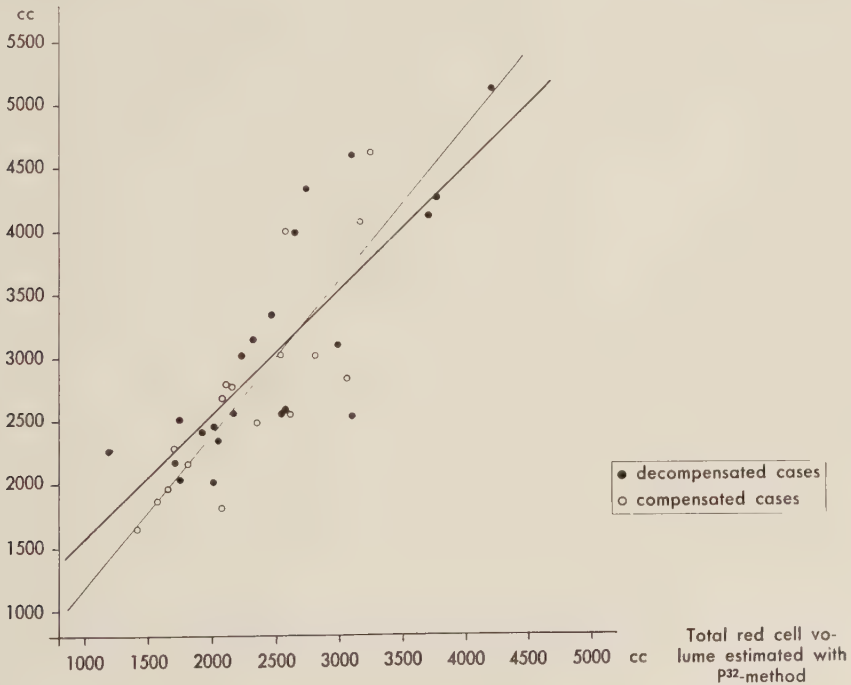


Fig. 6. Total red cell volume estimated by P^{32} and by Evans blue methods in decompensated and compensated cases of heart failure.

Fig. 6 gives the regression lines for total red cell volume estimated according to the Evans blue method and according to the P^{32} method. As seen from fig. 6, the regression coefficients differ somewhat for decompensated and compensated cases. Still, an analysis shows no significant difference ($b = 0.99 \pm 0.15$ and $b = 1.22 \pm 0.21$).

The means for the total red cell volume, as determined by the P^{32} and the Evans blue methods, are, in decompensated cases, 2508 cc and 3067 cc, respectively, and, in compensated cases, 2289 cc and 2741 cc, respectively. Accordingly, the P^{32} method has produced values for the amount of blood corpuscles in decompensated and compensated cases that are 18.2 and 16.5 per cent lower, respectively.

The "body hematocrit", i.e. the average hematocrit, has been estimated according to the following formula:

$$\frac{\text{Total cell volume (by } P^{32})}{\text{total cell vol. (by } P^{32}) + \text{plasma volume (by Evans blue)}}$$

The correlation between "body hematocrit" and venous hematocrit in the 22 decompensated cases will be seen from fig. 7.

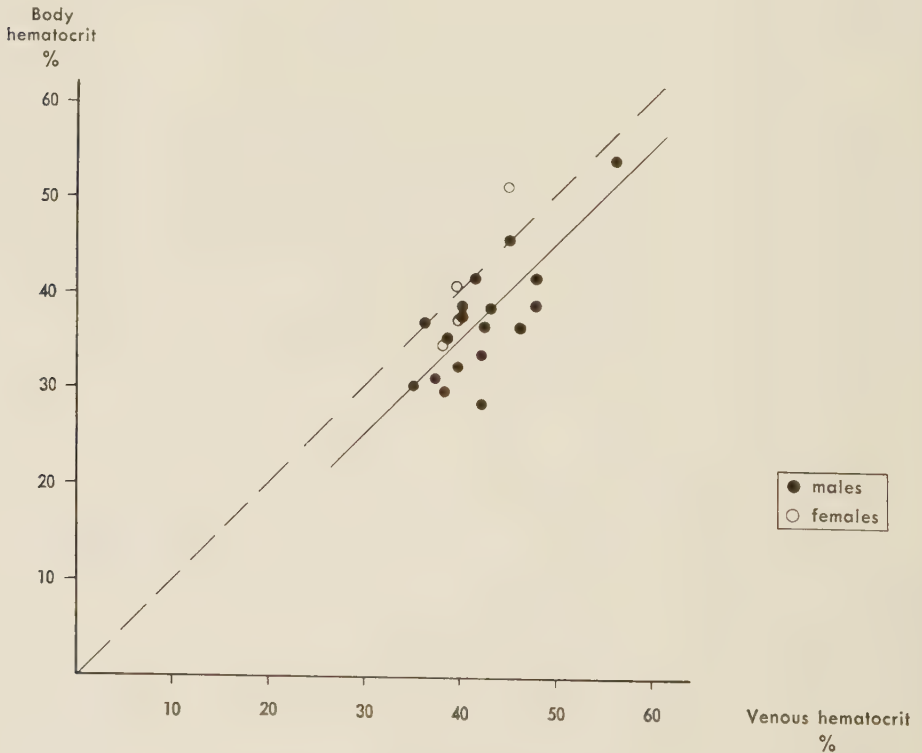


Fig. 7. Relationship between venous hematocrit and body hematocrit in congestive heart failure.

It appears that the "body hematocrit" is lower than the venous hematocrit. On an average, the quotient between body hematocrit and venous hematocrit amounts to 0.90 in decompensated cases.

CIRCULATION IN DIFFERENT VASCULAR AREAS

In 3 severe cases of congestive heart failure, blood samples were with-drawn simultaneously from the brachial artery, the brachial vein and in one case also from a crural varicose vein — representing a stagnant vascular area — after injections of labelled corpuscles and the blue azo dye.

The various activities of the corpuscles and the extinction values of the dye in plasma are plotted against time in *fig. 8*, taken from one of the forementioned cases.

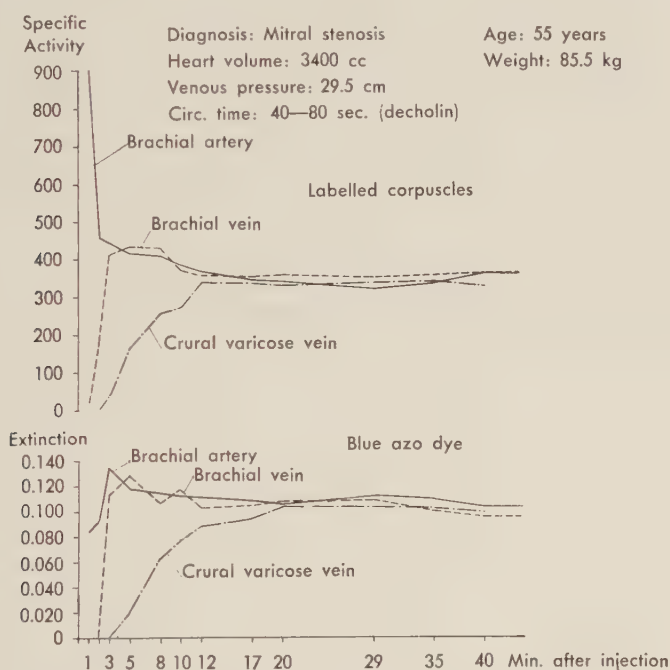


Fig. 8. Mixing conditions of labelled corpuscles and blue azo dye in a case of congestive heart failure.

When studying only the activity curves of labelled corpuscles from different vascular areas, it will be seen that some time will elapse (about 5 minutes in this case) before the impulses from the brachial venous samples equal those of the arterial samples. There is a marked delay in the corpuscular activity of samples drawn from the crural varicose veins, it attains the same value as those from the arterial and venous samples at the 12th minute. In the two other cases the impulses from the brachial venous samples equal those of the arterial samples in the 4th and 8th minutes respectively.

In comparing the extinction values of the dye from the brachial artery and those from the brachial vein, they seem approximately to coincide in time with the corpuscular activity from the same vessels, (this being true of all three cases). In a comparison of the circulation in stagnant vascular areas, e.g. crural varicose veins, the extinction values of the dye from arterial and varicose venous blood will be observed to agree later than the corresponding values of the corpuscular activities, but this can be due to a mere chance.

Influence of work.

In 6 cases of congestive heart failure, the patients performed a certain amount of muscular work during the investigation. Owing to their fairly severe congestion the exercise of the patients has to be limited. The patients had to walk about for 4–10 minutes in the room. The exercise test was in general performed between 40–55 minutes. Before and after several venous blood samples were withdrawn.

In the investigations of these 6 cases, no statistical difference was ascertained as regards corpuscular activity before and after work.

In every case the samples were subjected to simultaneous tests as to their content of blue azo dye. A line representing the disappearance was drawn from the values of the samples taken before work. The extinction values of the dye from samples after work will be seen to be definitely higher than might have been expected, judging from the disappearance slope. This was noted in each case.

DISCUSSION

From the present investigation, as well as from the survey of literature, there is reason to suppose that the rate of circulation varies in different vascular areas. To conclude from "dilution curves" from arterial blood, where the samples are collected at very short intervals, the labelled corpuscles will, however, fairly rapidly be mixed in the large vascular areas (NYLIN). It would, probably, be very difficult to determine simply from the arterial "dilution curve" when such a mixing has occurred in cases of congestive heart failure, seeing that "re-circulation" changes the curve (NYLIN). When, on the other hand, comparing the activity in drawn samples from the brachial artery and the brachial vein, it should, in cases of cardiac insufficiency, be reasonable to assume that a mixing has taken place in this vascular area within 10 minutes. On the other hand the circulation is, delayed in vascular areas such as in a stagnant varicose venous system. (In an observed case, mixing seemed to be approximately completed 12 minutes after the injection. In this case coinciding activity values were already obtained in the 5th minute in the arterial and venous blood).

Other vascular areas may conceivably show an even more reduced rate of circulation. They are, however, in all likelihood not of a size to affect, in any noteworthy degree, the appearance of the curve of corpuscular activity in venous blood, supposing that due regard is paid to the error inherent in every drawn blood sample. Nor are the blood

corpuscles likely to have been separated in blood depot organs of any notable importance. In favour of this, is the fact that mild exercise, in the performed experiments failed to cause any significant change in the corpuscular activity of the drawn samples. In this instance, the exercise tests in the present investigation conform to the results recorded by NYLIN and others with an isotope technique in normal cases.

Observations in investigations of the content of radio-Fe labelled erythrocytes in large veins and perfused organs, by GIBSON, SELIGMAN, PEACOCK, AUB, FINE and EVANS (1946), previously quoted, are also in keeping with the forementioned results. The investigators, just referred to, concluded from their findings that all blood corpuscles in the vascular system are in an active circulation. This seems to be suited to the purpose of the erythrocytes, viz, to supply oxygen to the tissue.

There is, to conclude from the literature, reason to suppose that the mixing is slower for plasma than for blood corpuscles. It should also be more difficult to decide whether all the plasma is in active circulation. It falls outside the scope of the present study to enter into this problem. The peculiar findings after exercise of increased extinction values of Evans blue remain to be explained. The question will, no doubt, arise whether muscular work that has been performed about 50 min. after the injection does not accelerate the return of blue azo dye to the circulation from the lymph to which it has rapidly disappeared after the injection. From this, it may seem likely that the concentration rises in the plasma after exercise.

The observed difference between body and venous hematocrit is in agreement with those reported in the literature. The explanations of this difference has already been surveyed. It appears, accordingly, natural to determine the total red cell volume by means of cell volume methods.

CHAPTER VIII

Bone marrow in cardiac insufficiency

The appearance of the sternal marrow has been investigated in 14 cases of cardiac insufficiency. 8 cases are due to vascular, and 6 to valvular affections.

ERYTHROPOIESIS

The erythropoiesis is characterized by signs of retarded and disturbed maturity. Anisocytosis and polychromasia are noted, as well as, occasionally, basophilic dots, particularly in orthochromatic erythroblasts. Nuclear deformations and occasional Howell-Jolly bodies occur.

Table 12. Sternal marrow in congestive heart failure (differential count of 2000 cells), as well as in normal cases.

	Total	Vascular heart diseases	Valvular heart diseases	Normal cases	
Reticul. cells	10,6 ± 1,4 (14)			{ 12,4 c) 13,1 d) 14,1 e) 17,8 f) 22,0 g)	
Erythropoietic cells	21,7 ± 1,9 (14)	20.8 (8)	22.3 (6)	a)	b)
proerythroblasts		0.6	0.7	0.5	2.2
basophil. erythroblasts		2.4	2.4	1.1	3.1
polychrom. erythroblasts		12.2	12.4	15.6	11.0
orthochrom. erythroblasts		5.6	6.9	0.7	1.5
Myelopoiet. + Lymphocyt. + Monocytoïd cells	67,6 ± 2,6 (14)				

$\bar{x} \pm \varepsilon_{\bar{x}}$ mean ± error of mean
The values are (n) = (number of cases)

Normal cases:

- a) acc. to Nordenson, 1951
- b) acc. to 26 authors compiled by Nordenson
- c) acc. to Segerdahl, 1935
- d) acc. to Ott, 1939
- e) acc. to Young & Osgood, 1935
- f) acc. Scott, 1939
- g) acc. to Wintrobe, 1942.

The result of a differential count of 2.000 nucleated cells will appear from *table 12*, where changes in the erythropoiesis are chiefly noted.

The table includes values of the erythropoiesis from different normal materials (SEGERDAHL, NORDENSON, OTT, YOUNG & OSGOOD, SCOTT, WINTROBE, as well as a normal material compiled by Nordenson from 26 other authors). An earlier report on bone marrow findings in normal cases was presented by C. M. PLUM in 1941. Since different authors derive their material from different populations — varying in diverse respect even as regards sex and age composition — it is no matter of surprise to find fairly considerable variations in the magnitude of the normal values. A comparison with a pathologic material will therefore not be quite reliable.

In the present material of cardiac insufficiency, the number of nucleated red cells amounts, on an average, to 21.7 per cent, with apparently congruous values for vascular and valvular heart affections. This value is moreover comparatively higher than the normal values stated by SEGERDAHL, OTT, YOUNG & OSGOOD, conforming also fairly well with OTT's value, viz, 23.4 per cent, from an equally large material of cardiac insufficiency. Thus, there is reason to assume that an actual difference exists.

SEGERDAHL gives the normal values for men and women under 30 years of age, the latter being numerically lower, though not differing significantly from that of the men. In a mixed material, including men and women over 60, SEGERDAHL obtained a normal value that lies between the two forementioned groups.

Nor has C. PLUM, on a material of 40 cases being able to detect any sure relation between age and cells belonging to the erythropoiesis from the 18th to the 70th year of life.

Obviously, the variations, as expressed in figures, attributable to the age and sex factors, are comparatively small. For this reason, and considering further that a comparison between the present material and the normal values is a matter of judgment, the material of cardiac insufficiency has not been divided from the point of view of age and sex.

As to the percentage distribution of the various forms of erythroblasts, it seems broadly speaking, to be identical for valvular and vascular heart affections, though the valvular cases appear to display a higher percentage of orthochromatic erythroblasts than the vascular affections.

From the table, it further appears that the normal values of different erythroblasts vary rather considerably according to different investigators. From the data in the literature it is difficult to decide which material, among those used, will best correspond to that of the present author. Ultimately, this depends on the definitions of the various erythroblastic forms applied by the research workers. No comparison between the forementioned values and the present ones will, therefore, be made, apart from the orthochromatic erythroblasts, since, in this instance, the results of the various authors appear to be more in agreement. The present material of cardiac insufficiency possesses, in this respect, a relatively higher value. The resulting difference seems approximately to correspond to the recorded difference in the total figures of the erythropoiesis.

The percentage figures of the different erythroblasts in the individual cases are set forth in *fig. 9*.

The conformance between the courses of the individual curves seems strikingly good, especially as far as the vascular affections are concerned.

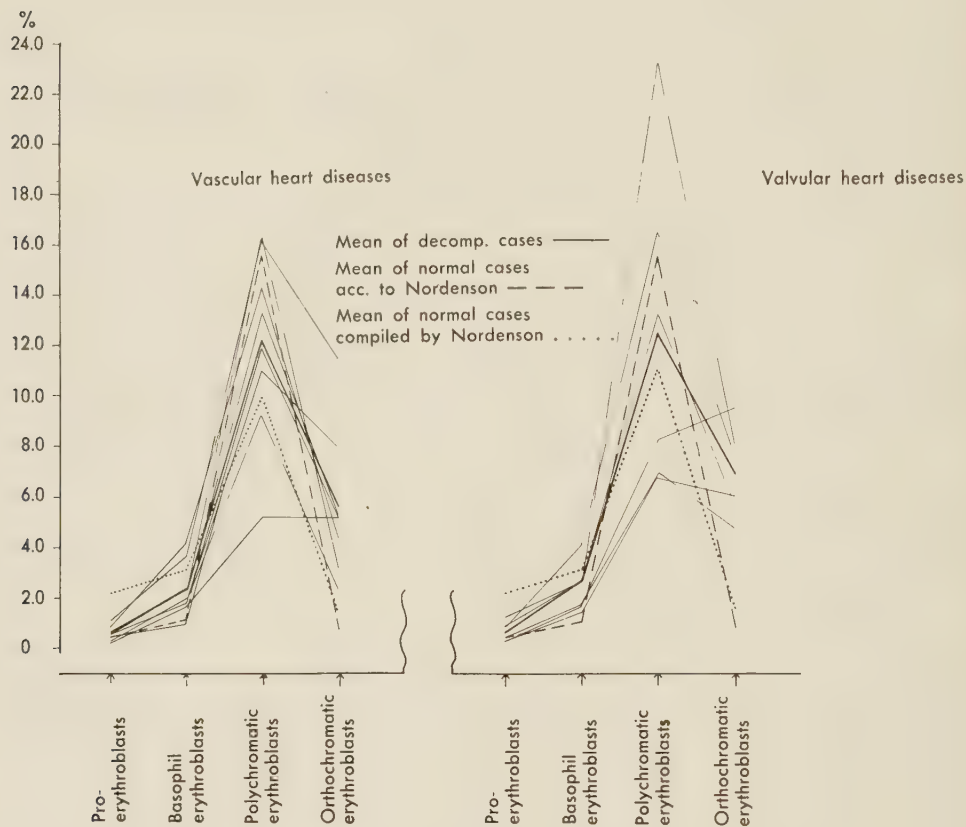


Fig. 9. Distribution of erythroblasts in congestive heart failure.

These means should furnish a comparatively good representation of the actual distribution of the erythroblastic forms in cardiac insufficiency.

In *fig. 10* and *fig. 11* the occurrence of macroforms and mitoses in the various erythroblastic stages is illustrated, for vascular as well as for valvular heart affections. Macroforms and mitoses are stated in percentages of the number of cells in the respective erythroblastic groups.

With regard to the vascular heart affections, the average mitotic figure shows a higher value for the basophilic erythroblasts and then successively decreases to polychromatic and orthochromatic erythroblasts. For the macroforms higher values will be observed in the proerythroblasts and in the orthochromatic erythroblasts and lower values in the basophilic and polychromatic erythroblasts.

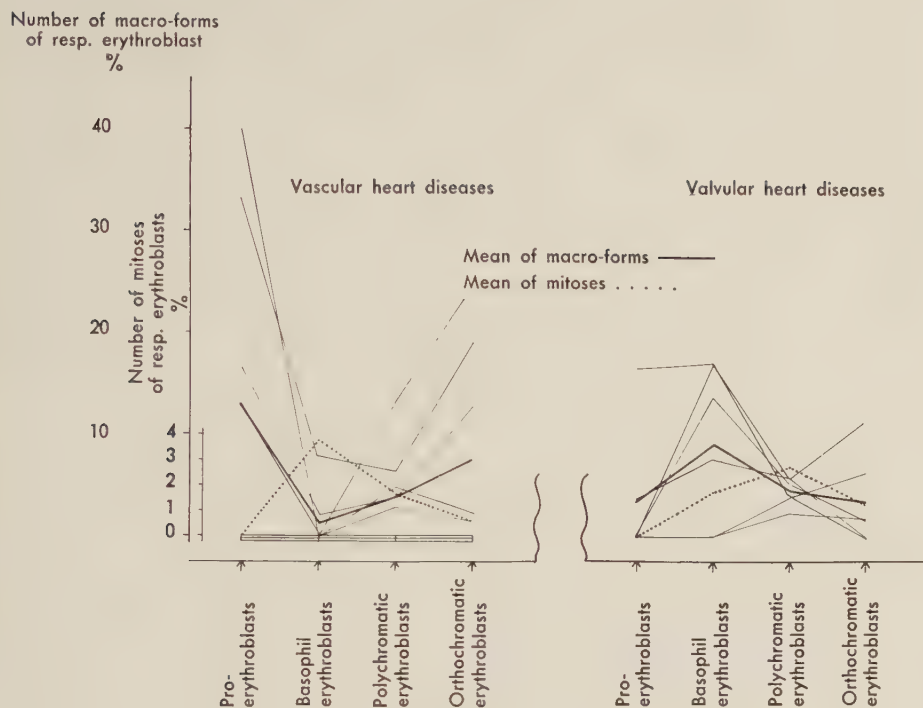


Fig. 10. Distribution of macro-forms in different erythroblasts in congestive heart failure.

In the valvular heart affections, on the other hand, the mitotic figure shows higher values for the polychromatic and orthochromatic erythroblasts. For the macroforms the highest value has been obtained in the basophilic erythroblasts.

The various curves show a fair conformity as regards the macro-forms and the mitoses. This should give a comparatively good conception of the actual distribution of macro-forms and mitoses among vascular and valvular heart failures, indicating that the erythropoiesis may disclose certain changes in these two types of heart affections.

Analysis of the material will show that a significant correlation exists between the percentage of nucleated red cells in the sternal bone marrow and the arterial oxygen saturation, the percentage value increasing with the degree of unsaturation ($r = 0.78 \pm 0.16$, $n = 6$, $t = 4.9^{**}$). No significant correlation has been found between the *MCD* of the polychromatic erythroblasts and the arterial oxygen unsaturation, ($r = 0.12 \pm 0.35$, $n = 6$, $t = 0.34$).

An almost significant correlation has been ascertainable between the total red cell volume and the number of nucleated red blood corpuscles ($r = 0.55 \pm 0.23$; $n = 9$; $t = 2.4^{*}$).

On the other hand it might still be added that one of the components in the last-mentioned correlation, viz, the total red cell volume, is determined not only by the inten-

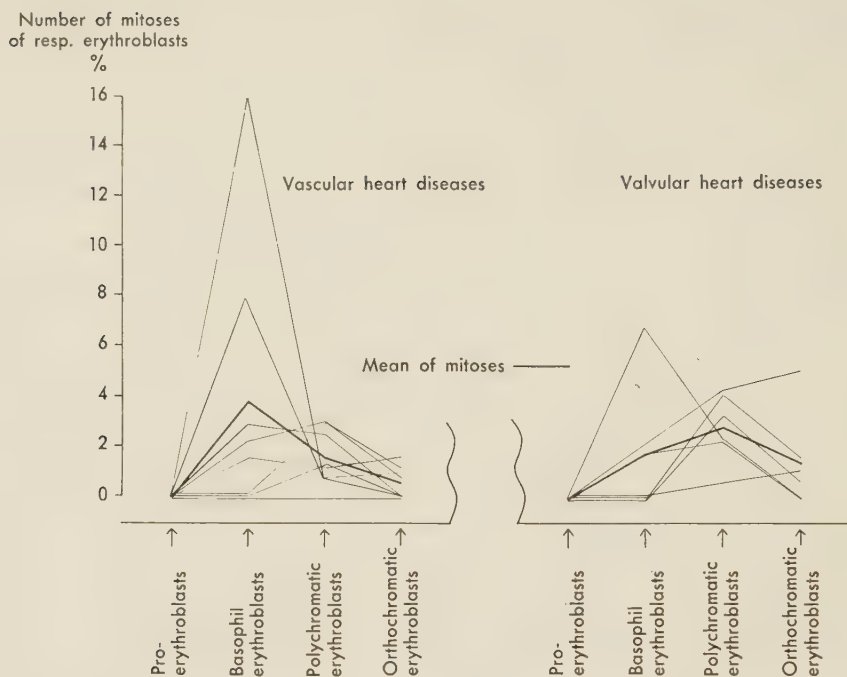


Fig. 11. Distribution of mitoses in different erythroblasts in congestive heart failure.

sity of the production of blood corpuscles but also by the degree of destruction. It should further be added that these percentage figures need not necessarily be an expression of the functional capacity of the erythropoiesis.

In the survey of the literature, theories have been quoted to the effect that the mature erythrocytes develop either from the orthochromatic or from the polychromatic erythroblasts. In the event of a more marked erythropoiesis, it is, possible that erythrocytes develop directly from both of these preliminary stages. Supposing this to happen, the *MCD* of the erythrocytes in the peripheral blood might be expected to some extent to be correlated to the quotient of orthochromatic/polychromatic erythroblasts in the bone marrow. In this connection, however, it should be borne in mind, that, in both forms of erythroblasts, there are macroforms to be found.

When tested on the basis of the present material of cardiac insufficiencies, the correlation between a quotient of the following appearance

$$\frac{\% \text{ of macroforms of orthochromatic erythroblasts}}{\% \text{ of macroforms of polychromatic erythroblasts}}$$

and the *MCD* of the erythrocytes in the peripheral blood will be found to be significant ($r = 0.66 \pm 0.17$; $n = 11$ $t = 3.8^{**}$).

The regression of the observed correlation is reproduced in *fig. 12*. From this, the *MCD* will be seen to increase with a rising value of the quotient.

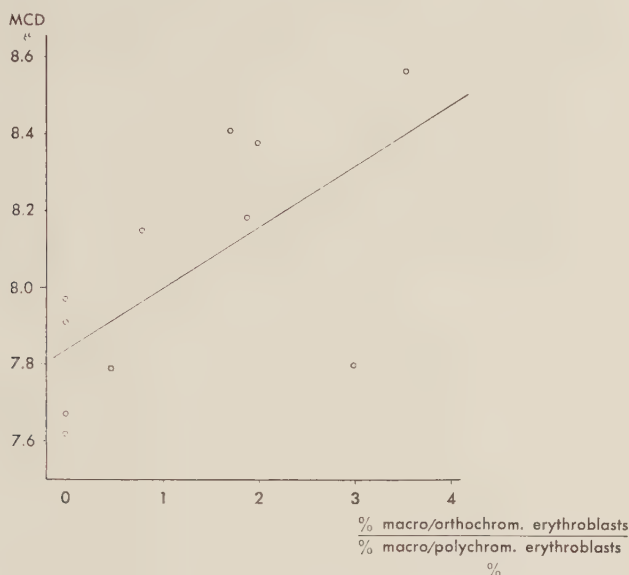


Fig. 12. Relationship between $\frac{\text{orthochromatic}}{\text{polychromatic}}$ erythroblasts and *MCD* of red blood corpuscles in peripheral blood.

No correlation has been obtained between the *MCD* of the erythrocytes and the *MCD* of the polychromatic erythroblasts ($r = 0.14 \pm 0.30$, $n = 11$, $t = 0.47$).

In the case of cardiac insufficiency a significant correlation has been observed between the quotient of $\frac{\text{orthochromatic}}{\text{polychromatic}}$ erythroblasts and the *MCHC* of the erythrocytes ($r = 0.59 \pm 0.19$, $n = 12$, $t = 3.2^{**}$), while no significant correlation has been ascertainable ($r = 0.14 \pm 0.28$) between the quotient and the *MCH* of the red blood corpuscles in the peripheral blood. (*Fig. 13*).

MYELOPOIESIS

In the myelopoiesis, retarded maturity is, as a rule, to be noticed, with a relative increase of immature cells and signs of disturbed maturity with toxic granulations. In addition, a certain increase of monocytoïd cells and lymphocytes is not seldom to be found, partly with outflowing plasma. Fairly often, an increase of eosinophil cells will be observed.

As will appear from *table 12* the average total of myelopoietic and monocytoïd cells, together with lymphocytes, amounts to 67.6 per cent.

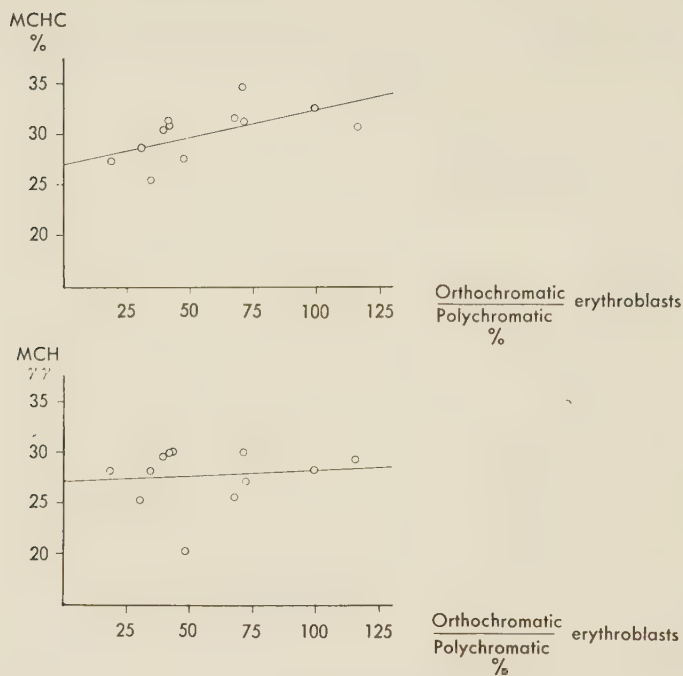


Fig. 13. Relationship between $\frac{\text{orthochromatic}}{\text{polychromatic}}$ erythroblasts and MCHC respectively MCH of red blood corpuscles in peripheral blood.

CHAPTER IX

Total red cell volume in congestive heart failure

I. GENERAL SURVEY

In altogether 55 decompensated cases with valvular and vascular heart diseases, accounted for in earlier chapters, the total red cell volume has been determined by the previously stated method. Then, repeated determinations have followed in the course of the treatment. The total red cell volume has also been calculated per m^2 of body surface. For the calculation of body surface, such a value of the weight has been applied as has been available when the patient has been, as far as possible, free from edema. It must be misleading to correlate the total red cell volume to body weight when varying degrees of edema are present.

Data of the total red cell volume for men and women, in valvular and vascular heart affections, during decompensation and compensation, will be found in *table 13*, together with data from the control groups.

From the table it will be seen that, generally speaking, the red cell volume, totally as well as calculated per m^2 of body surface, is higher in decompensated than in control cases, higher in decompensated than in compensated cases, higher in men than in women, and higher for valvular than for vascular heart affections. It should further be noted that in the valvular groups, as well as in the group of men with vascular heart diseases the dispersion is much greater than in normal cases and that, obviously, the morbid condition has affected the dispersion more than the means. The explanation might, conceivably, be that for some individuals the total red cell volume has markedly increased, while, in other cases, it has remained practically normal.

When comparing the sexes, the dispersions are, in both groups of heart affections, found to be much greater among men than among women. This applies also to normal cases, though not so conspicuously.

The statistical tests, the *t*-analyses, have been performed from red cell volume per m^2 of body surface, since a correlation is found between total red cell volume and body surface. This correlation should, as a matter of course, be eliminated, in order to prevent its appearance as a variation factor. This correlation could be dispensed with by adopting predicted values of some other kind, satisfactory from a statistical point of view, but cc/m^2 of body surface appears in practice to be the most convenient.

Table 13. Total volume of erythrocytes in decompensated and compensated heart failure as well as in control cases.

	Total amount of erythrocytes cc			Amount of erythrocytes/body surface cc/m ²		
	Decompensated	Compensated	Control	Decompensated	Compensated	Control
Males	{ Vascular heart diseases }	2444 ± 84 461 (30)	2164 ± 70 337 (23)	2166 ± 56 331 (35)	1191 ± 42 201 (23)	1131 ± 22 131 (35)
		2721 ± 282 797 (8)	2662 ± 316 546 (3)	1517 ± 132 373 (8)	1421 ± 158 274 (3)	
Females	{ Valvular heart diseases }	1783 ± 74 196 (7)	1595 ± 186 416 (5)	1119 ± 43 113 (7)	983 ± 114 255 (5)	
		2119 ± 185 585 (10)	1715 ± 100 245 (6)	1345 ± 114 360 (10)	1042 ± 56 137 (6)	868 ± 44 115 (7)

The values are $\bar{x} \pm \epsilon_{\bar{x}}$ mean $\pm$ error of mean
 s (n) standard deviation (number of cases)

In all groups, a *t*-analysis will show a significant difference between the decompensated and the control cases. Likewise, an almost significant difference between decompensation and compensation can be ascertained in total red cell volume in men with vascular heart affections and in women with valvular defects, while, in the two other groups, no significant differences are traceable. The latter fact may be due to the relatively small number of cases.

Between compensated and normal cases only one difference is seen to be almost significant, namely between normal females and females with valvular heart defects. In the comparison between decompensated groups of males and females and between vascular and valvular heart diseases, only one difference has been proved to be significant, namely that between males and females with vascular heart diseases. (*Table 14*).

Table 14. Differences in means.
(Total red cell volume cc/m² body surface.)

		Decomp. — normal	Decomp. — comp.	Comp. — normal
Vascular heart diseases	males	199 ± 52 3.83*** (65)	139 ± 63 2.21* (53)	60 ± 47 1.28 (58)
	females	251 ± 62 4.05*** (14)	136 ± 122 1.11 (12)	115 ± 122 0.94 (12)
Valvular heart diseases	males	386 ± 134 2.88** (43)	96 ± 206 0.47 (11)	290 ± 160 1.81 (38)
	females	477 ± 122 3.91** (17)	303 ± 127 2.39* (16)	174 ± 71 2.45* (13)
		Valvular — vascular heart diseases		Males — females
Decomp. males		187 ± 140 1.3 (38)	Valvular heart diseases decomp.	172 ± 174 0.99 (18)
Decomp. females		226 ± 122 1.85 (17)	Vascular heart diseases decomp.	211 ± 64 3.3** (37)

$$\begin{array}{l} \text{The values are} \\ \frac{d \pm \epsilon_d}{t(n)} = \frac{\text{difference} \pm \text{error of difference}}{t = \frac{d}{\epsilon_d} \text{ (number of cases)}} \end{array}$$

It might be added that a rather high total red cell volume was obtained in the case of chronic cor pulmonale. It amounted to 4400 cc, respectively 2430 cc/m² body surface. On compensation the observed corresponding values were 3180 and 1760 respectively. Any further studies on this case have not been performed.

II. SOME CLINICAL FACTORS INFLUENCING THE TOTAL RED CELL VOLUME

Some clinical factors that may exert influence on the total red cell volume during decompensation have been closely observed. They include the duration of congestion, the age of the patients, the degree of the congestion, the heart volume and the arterial oxygen saturation.

The criteria applied for the ascertainment of the duration of a congestion up to the time of the examination, as well as for determining the actual degree of a decompensation, have been stated earlier.

Age and duration of congestion.

The average value of the duration of insufficiency in the observed cases of vascular heart diseases is for males 3 years and for females 4 years. Corresponding values in valvular heart defects are found to be $6\frac{1}{2}$ and $6\frac{1}{2}$ years, respectively.

Evidently, manifest symptoms of insufficiency in vascular heart affections have, on the whole, been of a rather short duration. In the valvular cases, the duration has been more prolonged.

It is, likewise, obvious from the *table 11* that the mean age for the decompensated valvular heart affections is considerably lower than for the vascular. This age difference is typical, having regard to the genesis and development of the both heart affections.

No correlation between the total red cell volume and the two other variables (age and duration of insufficiency) proved ascertainable, either by simple or multiple regression analyses of the vascular group. All analyses were applied to men of the insufficiency group I, since this group is the only one with a satisfactory range of duration of congestion.

In the valvular group of insufficiency, a highly significant correlation between the total red cell volume and the duration of the insufficiency ($r = 0.89 \pm 0.08$ $n = 8$; $t = 11.1^{***}$) is noted for men. This correlation differs from the corresponding figure for men with vascular heart affections ($r = 0.35 \pm 0.21$; $n = 18$; $t = 1.62$).

No correlation can be ascertained for women with valvular heart affections ($r = 0.27 \pm 0.29$ $n = 10$). A close study of this female group will, however, show that in all cases, except one, the distribution in a bivariate regard, keeps uniform to that of the men. The single exception has considerably affected the correlation in an unfavourable direction. Nor is it reasonable to expect that women should reveal a behaviour, differing from that of men, with regard to the correlation of the total red cell volume to the duration of insufficiency.

It should be noted that these groups of valvular heart affections comprise all cases, irrespective of degree of decompensation when disclosing the same distribution in relation to the X -axis (insufficiency duration).

The regressions between total red cell volume and the insufficiency duration of both types of heart affections are to be found in *fig. 14*. In this connection, no regard has been paid to the sex factor or the degree of decompensation.

The total red cell volume in the group of vascular heart affections will, not noticeably increase with the duration of insufficiency, while, simultaneously, it keeps above that of the control groups.

As to the valvular groups, on the other hand, the total red cell seems to be almost normal when the insufficiency is of a rather short duration, but gradually increases with

the duration of insufficiency, and finally, possibly reaches higher values than in the vascular cases.

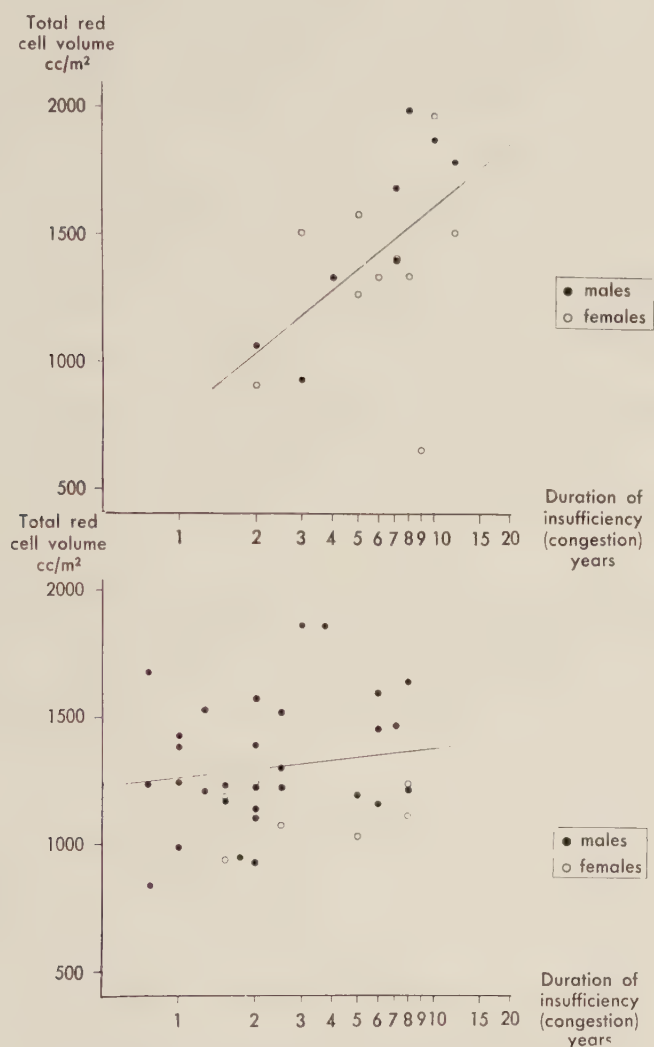


Fig. 14. Relationship between duration of congestion and total red cell volume in valvular and vascular diseases.

Degree of decompensation.

The degree of decompensation has been divided into 3 stages (groups), according to previously stated criteria.

The total red cell volume in different groups of decompensation will appear from table 15.

Table 15. Volume of erythrocytes (cc/m²) in vascular and in valvular heart diseases in different stages of decompensation.

	Insufficiency group I	Insufficiency group II	Insufficiency group III	Control group
males	1388 ± 67 283 (18)	1315 ± 80 211 (7)	1140 ± 55 124 (5)	1131 ± 22 131 (35)
Vascular heart diseases				
females	1100 ± 44 109 (6)	1240 (1)		868 ± 44 115 (7)
males	1905 ± 50 71 (2)	1359 ± 176 395 (5)	1750 (1)	
Valvular heart diseases				
females	1327 ± 143 403 (8)	1478 ± 93 131 (2)		

The values are $\bar{x} \pm s\bar{x}$ = mean ± error of mean
 s (n) = standard deviation (number of cases)

In the group of vascular heart affections (men), the total red cell volume is found to increase with a rising degree of insufficiency. A *t*-analysis discloses a highly significant difference between the insufficiency group I and the control group ($t = 3.62^{***}$), an almost significant difference between the insufficiency group II and the control group ($t = 2.22^*$) and no significant difference between the insufficiency group III and the control group ($t = 0.15$).

Thus, no difference of any practical importance seems to occur between normal cases and men belonging to the insufficiency group III, as far as the total red cell volume expressed in cc/m² of body surface is concerned. This is obvious from the noted, relatively inconsiderable, difference in means, as well as from the observed standard deviations that are approximately identical. Nor is, apparently, any more important difference to be recorded between the cases belonging to groups I and II. Between normal cases and cases in group III, on one hand, and those belonging to groups II and I, on the other, there is a difference, in so far as the last-mentioned cases show higher means as well as standard deviations. A *t*-analysis will, moreover, reveal a significant difference between the means for groups I and III. ($t = 2.85^{**}$). The comparison between groups II and III is, as a matter of course, of particular interest. True, no significant differences can be found either in means ($t = 1.81$) or standard deviations ($v^2 = 2.90$). But considering the relatively small number of cases comprising the material, the fact that the level of significance ($p = 0.05$) has almost been attained in both instances, implies that a difference exists between groups II and III with regard to the total red cell volume. In this connection it may be noted that, if such a pronounced difference occurs in the dispersions, it will also be found between the means.

Numerically, the increase in total red cell volume which occurs with a progressive degree of insufficiency, is, accordingly, largely confirmed by the more detailed statistical analysis.

Though big differences, as expressed in figures, can be observed between the group means, in the case of the valvular heart affections, they have not been shown to be significant, nor can any tendency, acceptable from a medical point of view, be traced in these values.

A progressive cardiac insufficiency is, *inter alia*, characterized by increasing edema and venous pressure. The influence of these two factors on the total red cell volume has been subjected to a special study.

The different degrees of edema have been divided into 3 subordinate groups, as previously defined. As usual the material has been classified according to diagnosis and sex. Corresponding values of the total red cell volume will appear in *table 16*.

Table 16. Volume of erythrocytes (cc/m²) in decompensated heart failure in different stages of edema.

		Edema ++ or + - + +	Edema + +	Edema + or -
Vascular heart diseases	males	1437 ± 67 241 (13)	1272 ± 87 274 (10)	1210 ± 83 219 (7)
	females	1053 ± 72 125 (3)	1169 ± 52 105 (4)	—
Valvular heart diseases	males	1780 (1)	1510 ± 114 161 (2)	1465 ± 177 396 (5)
	females	1302 ± 162 429 (7)	1500 (1)	1420 ± 29 40 (2)

The values are $\bar{x} \pm \epsilon \bar{x}$ = mean ± error of mean
 s (n) standard deviation (number of cases)

Also in this instance, the total red cell volume shows a tendency to increase with the degree of edema in men with vascular heart affections. An almost significant difference in the total red cell volume has been ascertained between the groups with the greatest and least degrees of edema ($t = 2.12^*$).

With regard to the other groups, it is sufficient to note that the total red cell volume, in the case of men with valvular heart defects, increases with a rising degree of edema, but that this may be accidental, seeing that, here, the mean errors are predominant, as compared to the differences.

It should be mentioned that, on the other hand, no correlation between total red cell volume and venous pressure has been observable for either cases with vascular heart failure or cases with valvular heart defects.

Heart volume.

The heart volume, as far as it has been roentgenologically ascertainable in the present material, will appear from *table 17*. It includes normal values from an unpublished material by NYLIN.

Table 17. Heart volume in congestive heart failure as well as in normal cases.

	Males	Females
Vascular heart diseases	1832 $\pm$ 105 483 (21)	1353 $\pm$ 116 201 (3)
Valvular heart diseases	2542 $\pm$ 246 697 (8)	1924 $\pm$ 204 579 (8)
Normal cases acc. to Nylin	740 $\pm$ 9 134 (240)	571 $\pm$ 7 97 (181)

The values are $\bar{x} \pm \epsilon \bar{x}$ = mean $\pm$ error of mean
 s (n) = standard deviation (number of cases)

The heart volume will be found to be several times normal size. Also, it seems to be bigger in the valvular than in the vascular heart affections. Decompensated men, as well as normal, show a bigger heart volume than decompensated, or normal, women.

t-analyses show that the observed numerical differences in most comparisons display some degree of statistical difference.

In the case of normal men, a correlation has already been found to exist between the total red cell volume and the heart volume ($r = 0.67 \pm 0.13$ $n = 17$ $t = 5.15^{***}$). Considering the marked changes in the heart volume and the total red cell volume that occur in a material of cardiac insufficiency, the heart volume increasing particularly in mean and the total cell volume in dispersion, it is interesting to observe if a correlation will continue to appear.

The size of the materials has permitted an analysis of only three of the groups of cardiac insufficiency. An almost significant correlation coefficient ($r = 0.57 \pm 0.24$, $n = 8$ $t = 2.36^*$) has been noted only in one of these three groups, viz, that of men with valvular heart defects.

Arterial oxygen saturation.

Arterial oxygen saturation in decompensation and compensation, respectively, for vascular and valvular heart affections, will be found in *table 18*. Normal values have been quoted to vary from 95% to 99%.

Table 18. Arterial oxygen saturation in decompensated and compensated cases of heart failure.

	Decompensated	Compensated
Vascular heart diseases	90.9 $\pm$ 1.1 5.2 (21)	93.5 $\pm$ 0.9 3.4 (16)
Valvular heart diseases	93.2 $\pm$ 0.9 2.4 (8)	94.7 $\pm$ 1.1 2.7 (6)

The values are $\bar{x} \pm e_{\bar{x}}$ = mean $\pm$ error of mean
 s (n) standard deviation (number of cases)

The table shows slightly reduced saturation values in decompensated, as compared to normal and compensated, heart affections. According to the different values obtained in normal cases no statistical analyses will be made, as far as concern decompensated and normal cases. No significant difference has been found between decompensated and compensated cases, or between the two groups of heart affections.

The correlation to the total red cell volume has been tested, but neither for vascular nor for valvular decompensated heart affections has any significant correlation been observable.

Effect of treatment on total red cell volume.

In the course of the treatment of cardiac insufficiencies, repeated determinations of the total red cell volume have been performed. The material has been divided into two groups, one comprising cases that, through the treatment, have achieved compensation, or practically so, and another of cases that have improved but slightly or not at all during the period of observation. Only cases belonging to decompensation groups I and II form part of the material. Thus cases of group III have been excluded, on account of the fact that the clinical change, when passing from decompensation to compensation is, in their instance, but inconsiderable.

Since different values for normal men and women are noted, the material has been classified with regard to sex.

The values of the total red cell volume have been chronologically arranged for each individual, in weeks from the first determination. In cases when no weekly determinations have been made, the total red cell volume has been calculated by means of linear interpolation between adjacent observed values. On the basis of observed and "theoretical" values from all the individuals involved in this investigation, an average value for each week has been calculated. Then a straight line has been drawn adjusted to thus obtained weekly means, to describe the course of development.

In *fig. 15a* och *b* will be found the two lines that represent the course of the changes of the total red cell volume for men and women within the respective groups. In the group where a marked improvement has been obtained, the total red cell volume is seen to decrease during the period of observation, approaching normal values in the 6th week. In the group, on the other hand, where but a slight improvement, or none at

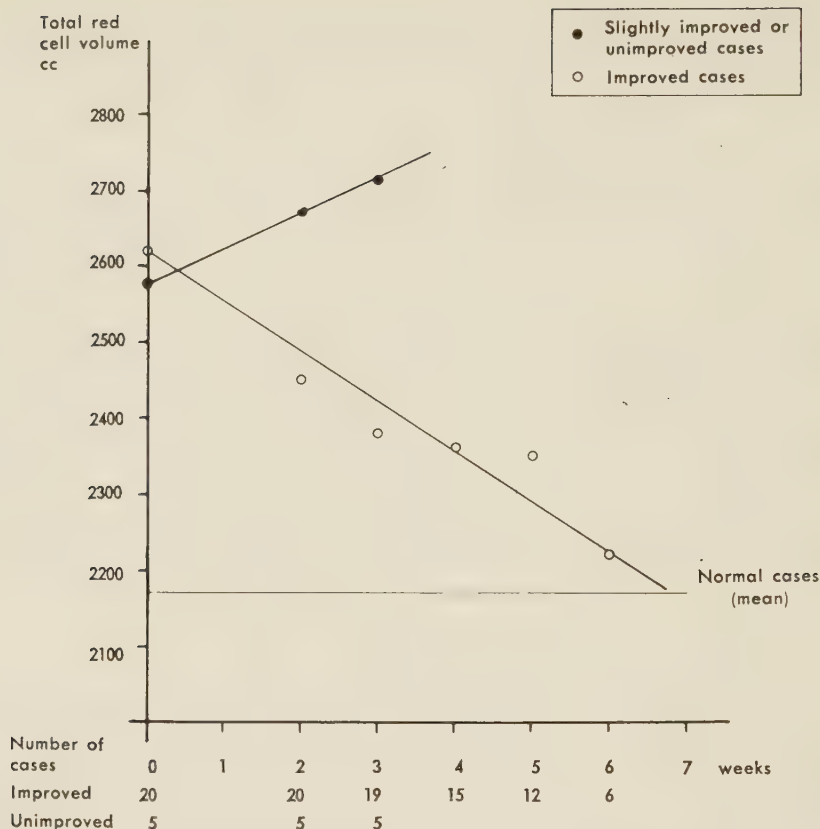


Fig. 15 a Changes in total red cell volume during treatment (Males).

all, has been achieved, the total red cell volume increases somewhat during the corresponding period. These observations apply to both men and women.

When comparing observed values for the total red cell volume during decompensation and compensation in the first-mentioned group, a significant mean difference (see table 19) will appear.

Table 19. Changes in total volume of erythrocytes after treatment of congestive heart failure.

	Improved	Slightly improved and unimproved
Males	-379 ± 84 4.51*** (20)	109 ± 145 0.75 (5)
Females	-459 ± 161 2.85* (9)	267 ± 454 0.59 (2)

$d = e_d$ difference $\pm$ error of difference
 The values are
 $t = \frac{d}{e_d} \cdot \sqrt{n}$ (number of cases)

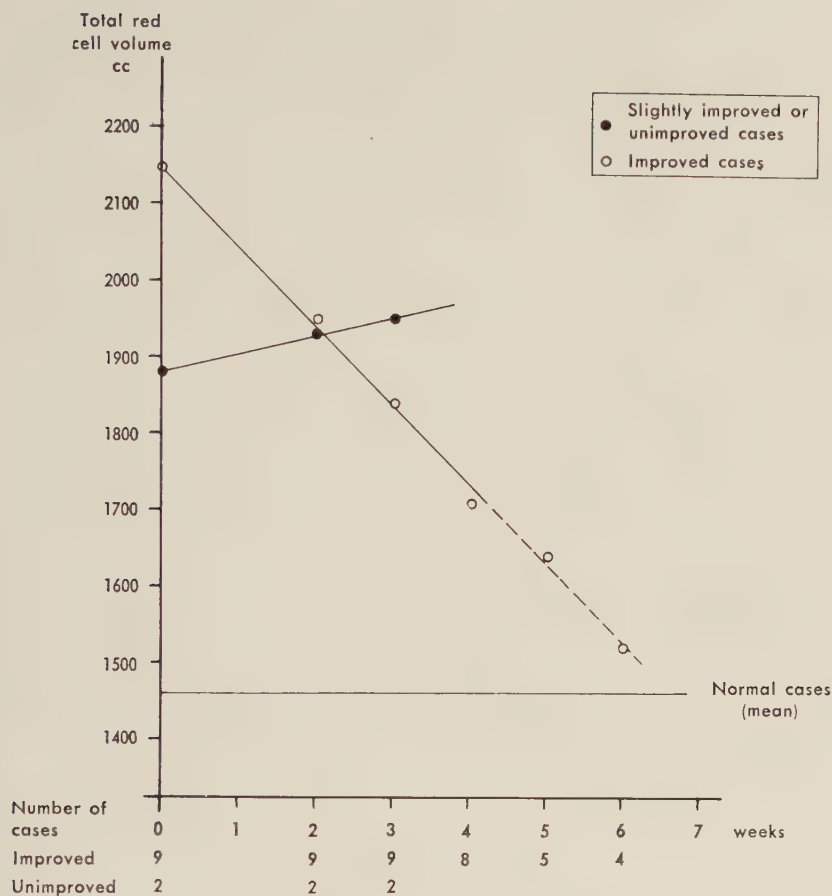


Fig. 51 b. Changes in total red cell volume during treatment (Females).

With regard to the other group, no significant change in the total red cell volume has been observed (see *table 19*). The analysis is founded on the differences between the first and last observed values for each individual.

CHAPTER X

The plasma volume in cardiac insufficiency

The plasma volume has been determined with Evans blue in a manner previously stated in 22 cases of cardiac insufficiency. The material has been arranged from the point of view of sex. The observed values in decompensation and compensation are to be found in *table 20*. In comparing the groups of men with vascular and valvular defects, no statistical difference could be demonstrated. No division into diagnostic groups has been made. In the table, also the normal values for the plasma volumes for men and women are recorded. They have been taken from VON PORAT's work (1951). This native material has been preferred for comparisons. It should, however, be mentioned that, from the point of view of method, the materials differ, in so far as PORAT has obtained his values by means of a linear extrapolation, a procedure that must be expected to yield somewhat higher values for a plasma volume than a semi-logarithmic extrapolation.

Table 20. Plasma volume in decompensated and compensated heart failure as well as in normal cases (estimated with blue azo dye).

	Decompensated	Compensated	Normal acc. to v. Porat (1951)
	cc.	cc.	cc.
Males	4350 $\pm$ 269 1110 (17)	3627 $\pm$ 173 628 (12)	3380 $\pm$ 120 534 (20)
Females	3319 $\pm$ 160 454 (5)	2913 $\pm$ 271 606 (5)	2681 $\pm$ 53 239 (20)

The values are $\bar{x} \pm \epsilon_{\bar{x}}$ mean $\pm$ error of mean
 $s \quad (n)$ standard deviation (number of cases)

From the table, higher values are found to occur in decompensation for both sexes, as compared to normal and compensation values, respectively. Also, compensated cases display higher values than the normal. In all groups, male values keep higher than those of the female cases.

By means of *t*-analyses, highly significant differences can be shown to occur between the decompensated and normal groups (*t* equalling 3.29*** for men and 3.78*** for women). The difference between decompensated and compensated cases is found to be

almost significant only for men ($t = 2.26^*$). The absence of a significant difference for women is probably due to the relative smallness of the compared groups. The numerical difference between compensated and normal cases cannot be proved to be significant either for men or women ($t =$ equalling 1.17 and 0.84 respectively). The difference between men and women can be ascertained as significant in all groups of comparison. The correlation between heart and plasma volumes in men has been studied, a highly significant correlation ($r = 0.82 \pm 0.09$, $n = 14$, $t = 9.1^{***}$) being recorded.

CHAPTER XI

The reticulocytes

In the course of treatment, determinations of the number of reticulocytes have, on an average, been performed 2—3 times per week. The reticulocyte curves obtained from the values have, as a rule, been found to disclose a distinct maximum after the hospitalization. This maximum is usually reached within the first week. Considering that the admission at the hospital has taken place more or less distantly from the onset of the actual decompensation, the individual reticulocyte curves will not be completely comparable. However, by adjusting the curves so as to make the peaks of the curves approximately coincide on the time axis, this imperfection will be largely overcome. Still, it should be borne in mind that an observed maximal point will practically always deviate, vertically and horizontally, from the corresponding theoretical position.

In some cases no curve maximum is noted. Instead, the curve will begin with a series of markedly falling reticulocyte values. In such instances, there is reason to suppose that the reticulocyte peak has been attained before the hospitalization. It is, however, impossible to know the actual value of the peak or when it occurred. In order to make the information in such cases serviceable for the present purpose, the highest observed value has been coordinated with the reticulocyte maxima of the more complete curves.

The material has been classified according to two principles, viz, vascular and valvular heart affections and clinically improved and unimproved cases. Accordingly, the individual curves are set forth in four groups, with both sexes taken together. This procedure is justified by the fact that no difference in the type of the curves between the two sexes can be observed and that, together, they will, therefore, hardly cause more than a small increase in the variation.

The group of clinically improved vascular heart insufficiencies will appear from *fig. 16*.

From this, a reticulocyte peak will be seen to occur during the culmination period of decompensation with a fairly considerable dispersion of the maximal values in the various individual cases. After but a couple of weeks, normal reticulocyte values are observed. The individual curves will show a rather congruous course with a limited dispersion of the values. After 3—4 weeks, an increase in the reticulocyte values by some percentages will again be found with a renewed increase in the dispersion.

Broadly speaking, the group of clinically improved valvular heart affections (*fig. 16*) reveals the same course.

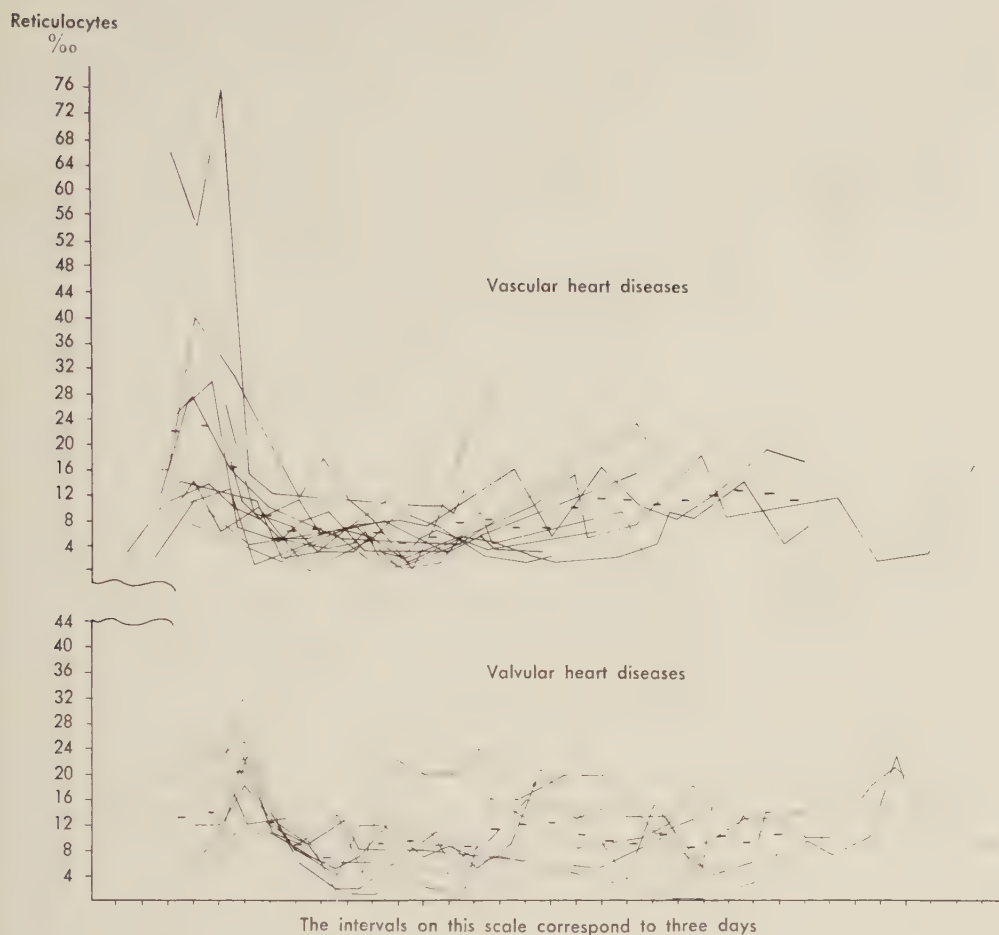


Fig. 16. Reticulocyte-curves during treatment of congestive heart failure (improved cases).

The clinically unimproved vascular heart affections are to be found in *fig. 17*.

Apparently, the individual curves keep, at the admission, at somewhat elevated reticulocyte values. Then they gradually diminish towards normal values, without showing any definite secondary peak.

The group of clinically unimproved valvular defects is to be found in *fig. 17*.

The reticulocyte values are somewhat elevated. They appear to vary in a casual manner during the time of observation. No tendency to a lowering of the reticulocyte values can be noticed.

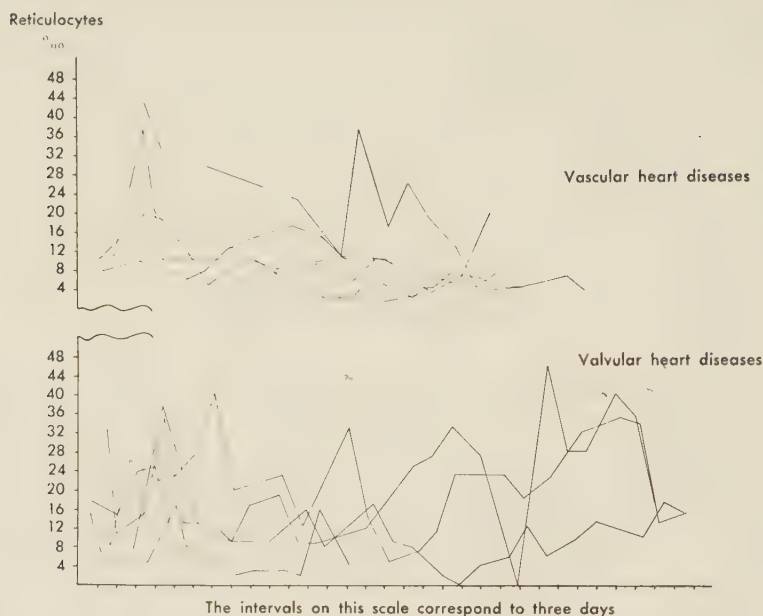


Fig. 17. Reticulocyte-curves during treatment of congestive heart failure (slightly improved or unimproved cases).

Table 21 gives the reticulocyte values for decompensation and compensation. The maximum of the reticulocyte curve has been chosen as a representative value of decompensation. It was considered appropriate to use this single value as the intervals between the tests were relatively so long as to render the means of successive tests generally less reliable. The lowest value of the reticulocyte curve, which usually is to be noted just before the second rise in the reticulocyte curve, has been adopted as a value of comparison in the case of a compensated condition. This procedure is justified by the fact that the minimum values of the different reticulocyte curves coincide approximatively in time, as well as by the small variation between the curves.

Table 21. Reticulocytes in decompensated and compensated heart failure.

	Decompensated	Compensated
Vascular heart diseases	23.1 ± 3.1	3.5 ± 0.7
	15.0 (24)	3.0 (21)
Valvular heart diseases	27.3 ± 4.1	3.9 ± 0.8
	16.3 (16)	2.7 (12)

The values are $\bar{x} \pm \varepsilon \bar{x}$ mean $\pm$ error of mean
 s (n) stand. dev. (number of cases)

From the table it will be seen that a slight to moderate reticulocytosis occurs during the decompensation and, further, that the means in vascular and valvular conditions of cardiac insufficiency conform. During compensation the reticulocyte values are normal.

The correlation between reticulocytosis and arterial oxygen saturation in decompensation was inquired into. The maximal value in decompensation was chosen as reticulocyte value. The correlation will be found in *fig. 18*.

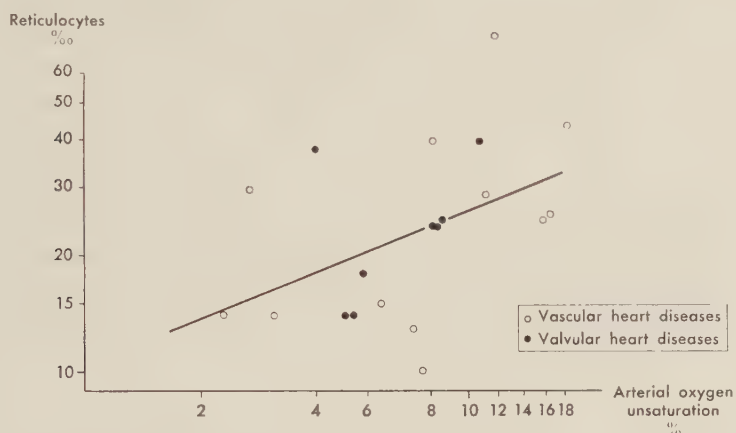


Fig. 18. Relationship between arterial oxygen unsaturation and number of reticulo-cytes.

An almost significant correlation is noted ($r = 0.46 \pm 0.18$, $n = 20$, $t = 2.62^*$). The correlation analysis is based on a material composed of men and women with vascular and valvular heart affections. According to close observations, the heterogeneity of the material has, on account of increased variation, rather reduced the correlation coefficient.

CHAPTER XII

The mean corpuscular diameter in cardiac insufficiency

I. GENERAL SURVEY

The 84 cases of cardiac insufficiency in which the *MCD* was determined were classified with regard to sex and the particular type of heart affection. The *MCD* was calculated both during decompensation and compensation, whenever attained. The materials of the groups thus formed will be found from *table 22*, together with values from a normal material, taken from GOLDBERG, GUNVALL, HAMMARSTEN & LINDGREN (1948).

Table 22. MCD in decomp. and comp. cases of heart failure as well as in normal cases.

		Decompensated	Compensated	Normal
Males	Vascular heart diseases	8.03 ± 0.04 0.26 (35)	7.84 ± 0.05 0.23 (24)	7.67 ± 0.02 0.14 (33)
	Valvular heart diseases	8.13 ± 0.06 0.22 (12)	8.20 ± 0.14 0.28 (4)	
Females	Vascular heart diseases	7.84 ± 0.05 0.15 (9)	7.76 ± 0.07 0.20 (8)	7.69 ± 0.02 0.13 (57)
	Valvular heart diseases	8.07 ± 0.05 0.26 (28)	7.81 ± 0.05 0.19 (14)	

The values are $\bar{x} \pm \epsilon_{\bar{x}}$ mean ± error of mean
 s (n) standard deviation (number of cases)

It will be seen from the table that the *MCD* in all four groups is higher during decompensation than normally. In three of the groups, the values will also be found to be, on an average, higher than during compensation. In comparisons within male and female group respectively valvular heart affections will be found to have higher values than the vascular cases. Further, the men show higher means than the women in the respective types of heart affection.

A *t*-analysis reveals that the differences in means between decompensated and normal cases are significant in all groups (see table 23).

Table 23. Differences in means. (*MCD* μ)

		Decomp. — normal	Decomp. — comp.	Comp. — normal
Vascular heart diseases	males	0.36 $\pm$ 0.05 7.20*** (68)	0.19 $\pm$ 0.06 3.20** (59)	0.17 $\pm$ 0.05 3.4** (57)
	females	0.15 $\pm$ 0.05 3.0** (66)	0.08 $\pm$ 0.09 0.9 (17)	0.07 $\pm$ 0.07 1.0 (65)
Valvular heart diseases	males	0.46 $\pm$ 0.06 7.66*** (45)	−0.07 $\pm$ 0.15 0.5 (16)	0.53 $\pm$ 0.14 3.78*** (37)
	females	0.38 $\pm$ 0.05 7.60*** (85)	0.26 $\pm$ 0.07 3.7** (42)	0.12 $\pm$ 0.05 2.4* (71)
		Valvular — vascular		Males — females
Decompensated	males	0.10 $\pm$ 0.07 1.43 (47)	Valvular heart diseases	0.06 $\pm$ 0.08 0.75 (40)
	females	0.23 $\pm$ 0.07 3.3** (37)	Vascular heart diseases	0.19 $\pm$ 0.06 3.2** (44)

$d \pm \epsilon_d$ difference $\pm$ error of difference.
 The values are $t = \frac{d}{\epsilon_d}$ (number of cases)

The same result has been obtained from the analysis of the differences between decompensated and compensated groups of men with vascular heart affections and women with valvular heart affections.

As to women with vascular and men with valvular heart affections, no significant difference has proved ascertainable between decompensated and compensated cases. This may well be explained by the comparatively more limited number of cases.

When comparing compensated and normal cases, significant differences of varying degree will be found in all the groups except that of women with vascular heart affections.

Within the sex groups, a significant difference between valvular and vascular heart affections can only be ascertained for women. As regards comparison between males and females a significant difference is found only in the vascular group.

II. SOME CLINICAL FACTORS INFLUENCING THE *MCD*

Duration of insufficiency.

The effect of the duration of insufficiency on the *MCD* of the erythrocytes has been studied in vascular and valvular heart affections in men as well as in women. In this

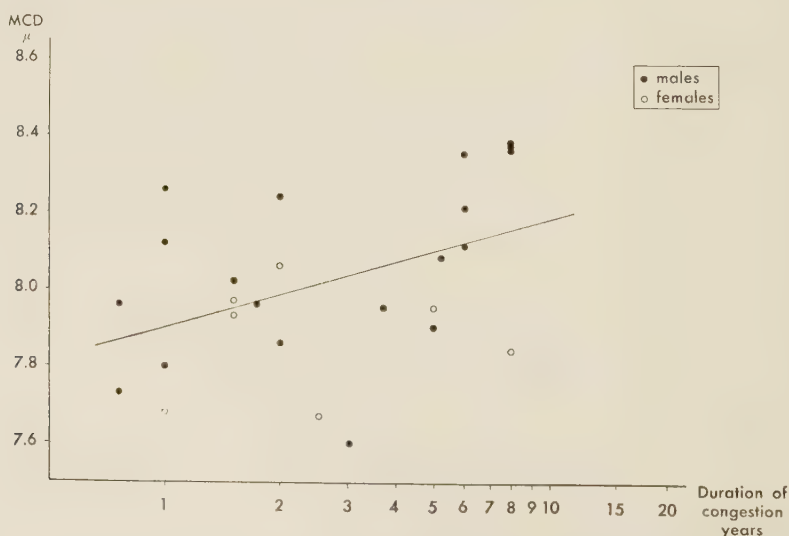
way, the material is divided into four groups. In addition, the influence of a pronounced decompensation has been regarded, inter alia, by excluding the insufficiency group III.

The groups of vascular heart affections comprise cases only from the insufficiency group I, since it shows a satisfactory range for duration of insufficiency from $\frac{3}{4}$ years up to 8, years, while cases, on the other hand, belonging to insufficiency group II have a range of from $\frac{3}{4}$ to $2\frac{1}{2}$ years. In the latter instance, no correlation can, as a matter of course, be noted between *MCD* and the duration of the insufficiency.

On the other hand, the groups of valvular heart affections comprise cases belonging to the insufficiency groups I and II, since, apparently, no differences in the horizontal dispersion, i.e. duration of insufficiency, occur. Vertically, i.e. the *MCD*, an increased dispersion may possibly be suspected, in consequence of the combination of insufficiency groups I and II. Because of this, an actual correlation, if any, between insufficiency duration and *MCD* may be more difficult to ascertain.

In the analyses, a significant correlation is noted between the *MCD* and the duration of insufficiency for men with vascular heart affections ($r = 0.51 \pm 0.17$, $t = 3.02^{**}$, $n = 19$), but not for women ($r = 0.11 \pm 0.37$, $t = 0.3$, $n = 7$). For women and men with valvular heart affections, no statistically significant correlations are, on the other hand, noticeable ($r = -0.24 \pm 0.19$, $t = 1.28$, $n = 25$) respectively ($r = -0.34 \pm 0.28$, $t = 1.21$, $n = 10$). The correlations noted here, may possible indicate, that an actual difference may occur between the two groups of heart diseases with regard to the change in the *MCD* in the course of the decompensation.

The regression between the *MCD* and the duration of congestion of vascular heart diseases is demonstrated in *fig. 19*. In this figure, no regard has been paid to the sex factor.



Age of patients.

As previously mentioned, the mean age is considerably lower in valvular than in vascular cases of cardiac insufficiency, though no correlation exists between the *MCD* and the age of the patients for either of the groups of congestive heart failure.

Degree of decompensation.

On the occasion of the examinations, cases of cardiac insufficiency, in which the *MCD* of the erythrocytes has been determined, have shown different degrees of decompensation. The influence of which on the *MCD* has been particularly studied. The results will be seen from *table 24*.

It can be demonstrated that, in the eight groups with the most pronounced symptoms of decompensation (insufficiency groups I and II), the means of the *MCD* significantly exceed the corresponding means in the normal material. The *MCD* means in the benign group of insufficiency do not differ from those of the normal materials except males with valvular heart diseases, where an almost significant difference can be obtained.

No definite course of development in the diameter values with an increasing decompensation is, however, ascertainable. It should, nevertheless, be noted that the insuffi-

Table 24. MCD in different stages of congestive heart failure as well as in normal cases.

		Insufficiency group I	Insufficiency group II	Insufficiency group III	Normal
Males	Vascular heart diseases	8.06 ± 0.05 0.23 (19)	8.06 ± 0.09 0.28 (9)	7.87 ± 0.11 0.28 (7)	7.67 ± 0.02 0.14 (33)
	Valvular heart diseases	8.05 ± 0.06 0.12 (4)	8.22 ± 0.11 0.26 (6)	8.01 ± 0.15 0.21 (2)	
Females	Vascular heart diseases	7.87 ± 0.06 0.15 (7)	7.79 (1)	7.63 (1)	7.69 ± 0.02 0.13 (52)
	Valvular heart diseases	8.07 ± 0.06 0.24 (17)	8.16 ± 0.10 0.27 (8)	7.85 ± 0.15 0.27 (3)	

The values are $\bar{x} \pm \varepsilon \bar{x}$ mean ± error of mean
 $s (n)$ standard deviation (number of cases)

ciency groups I and II, on one hand, and the insufficiency group III and the normal groups, on the other, display *MCD* means of the same order of size.

Further, when studying the relationship between the *MCD* and the various part symptoms of insufficiency, an increasing *MCD*, at a growing degree of edema, is found in the vascular, but not in the valvular heart affections (*table 25*). Still, from the point of view of significance, none of the differences between the edema groups are noteworthy.

Nor has any correlation between venous pressure and the *MCD* been ascertainable for either valvular or vascular heart affections.

Table 25. *MCD* in congestive heart failure at different degrees of edema.

		Edema + + or + + + +	Edema + +	Edema + or -
Vascular heart diseases	males	8.12 ± 0.07 0.26 (13)	8.00 ± 0.09 0.29 (12)	7.94 ± 0.09 0.29 (10)
	females	7.88 ± 0.06 0.12 (4)	7.84 ± 0.08 0.16 (4)	7.63 (1)
Valvular heart diseases	males	7.96 (1)	8.11 ± 0.10 0.23 (5)	8.17 ± 0.10 0.24 (6)
	females	8.08 ± 0.07 0.26 (14)	8.18 ± 0.09 0.23 (6)	7.97 ± 0.10 0.27 (8)

The values are $\bar{x} \pm \varepsilon_{\bar{x}}$ mean $\pm$ error of mean
 s (n) = standard deviation (number of cases)

Heart volume.

In the case of vascular and valvular heart affections, no correlation has been noted between heart volume and *MCD* either for men or women. It should, however, be borne in mind that, as compensation is reached, the means of both the heart volume and the *MCD* fall. This implies that, in a group composed of both decompensated and compensated cases, a correlation between heart volume and *MCD* should be expected.

Arterial oxygen saturation.

Nor has any correlation been ascertainable between the *MCD* and the arterial oxygen saturation for decompensated valvular and vascular heart affections.

Changes in the *MCD* with the clinical condition.

In 53 cases, belonging to the insufficiency groups I and II, the *MCD* of the erythrocytes in the peripheral blood has been studied, in the course of the treatment of the cardiac failure. Group III was excluded owing to the but slight clinical changes manifested at transition from decompensation to compensation.

Considering that, in the normal material, identical values have been noted for men and women, and, further, that in the event of cases of cardiac insufficiency being brought to practically complete compensation, i.e. to a normal condition, these normal values should be obtained, the material has not been classified with regard to sex in the present investigation of the influence of treatment on the *MCD* of the erythrocytes.

43 of the 53 cases have improved so considerably that clinical compensation has been reached or practically reached. In the other 10 cases, only a slight improvement has been noted during the observation time, or none at all.

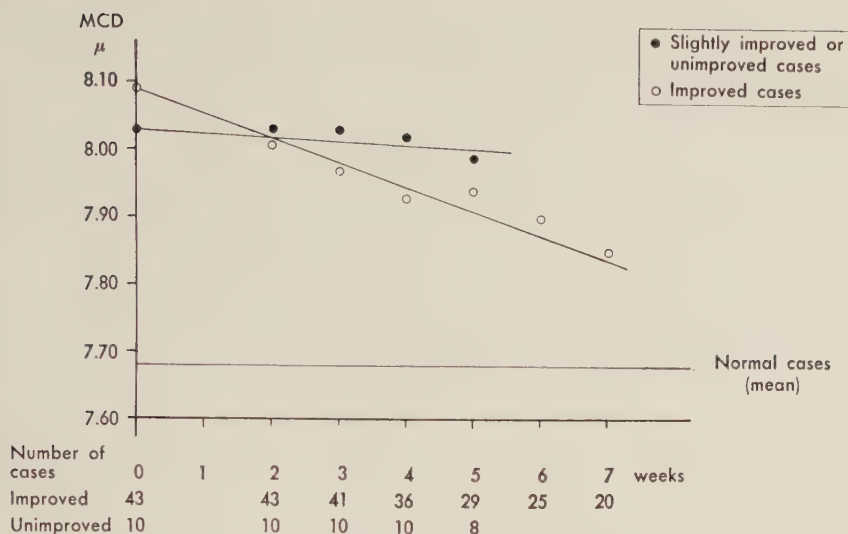


Fig. 20. *MCD*-changes during treatment of congestive heart failure (males and females).

The *MCD* values of each individual have been arranged in a chronologic order, stated in weeks from the first determination during the decompensation. For weeks when no determinations have been performed, the *MCD* has been calculated by means of linear interpolation between adjacent observed values. On the basis of observed and "theoretical" values from all individuals, an average value has been fixed for each week. Then, a straight line has been drawn, according to the average weekly values thus obtained, to represent the course of development.

Fig. 20 shows the gradual changes in the *MCD* in the two groups. In the figure, a line has also been inserted to represent the *MCD* in a normal material. The *MCD* decreases notably in the clinically improved cases, approaching normal values without attaining them during the observation period (7 weeks).

In a comparison between observed values of the *MCD* in decompensation and compensation in the individual cases of this group, a significant mean difference will be noted (table 26).

Table 26. Changes in *MCD* after treatment of congestive heart failure.

	Difference
Improved	-0.22 ± 0.03 6.4*** (43)
Slightly improved or unimproved	-0.10 ± 0.05 2.2 (10)

$$\text{The values are } \frac{d \pm \epsilon_d}{t} = \frac{\text{difference} \pm \text{error of difference}}{t} = \frac{d}{\epsilon_d} (\text{number of cases})$$

In the group of clinically slightly, or not at all improved cases, no significant diminution of the *MCD* will appear. The numerical decrease, however, seems well to correspond with the slight clinical improvement.

INVESTIGATIONS REGARDING THE CONDITION OF THE LIVER IN CARDIAC INSUFFICIENCY AND ITS CONNECTION WITH THE *MCD*

The liver stasis, a common form of stasis in cardiac insufficiency, originates in a back pressure. It appears early in a right ventricular failure. In a left ventricular failure, a liver stasis will gradually develop. Since the condition of the liver has been assumed to play a certain part in the genesis of macrocytosis, it would be useful to have access to a material of cardiac insufficiency, consisting of one group of unilateral left ventricular failure, and one of unilateral right ventricular failure, in order to shed light on this problem in cases of cardiac insufficiency.

The present material is, however, not suitable for such an analysis, since a palpable liver is to be found, to a large, and approximately equal, extent, in both vascular and valvular conditions of cardiac insufficiency, viz, about 93 per cent.

Liver tests.

The relationship between certain liver tests and the state of the heart decompensation, and the *MCD* of the red blood corpuscles respectively, has been subjected to further investigation.

The recorded values for bilirubin, phosphatase, citric acid, Takata, content of serum protein and the albumin-globulin quotient in these two groups of decompensated vascular and valvular heart affections are to be found in the table as well as normal values (*table 27*).

In addition, the table gives values from compensated heart affections. Since the bilirubin values are of a particular interest, regard has in the classification been paid to sex.

It should be noted that, because of the dilution of the plasma volume in cardiac insufficiency, the values recorded for bilirubin, phosphatase, citric acid and total protein are not quite comparable. Particularly during decompensation, the values are comparatively too low to be compared with corresponding normal values.

From the table, the bilirubin values are seen to be slightly elevated during decompensation, reaching normal values during compensation. It likewise appears from the table that the bilirubin values are higher in valvular than in vascular heart affections and higher in men than in women. By means of *t*-analyses, the observed differences between decompensation and compensation can be shown to be significant, except as far as vascular women are concerned. With regard to sex and groups of heart affections, respectively, the differences are not significant.

The noted values for citric acid and phosphatase during decompensation and compensation agree fairly well with the normal values stated in the literature.

Table 27. Some liver tests and total serum protein in decompensated and compensated heart failure as well as in normal cases.

	Bilirubin mg %		Phosphatase E./100 ml	Citric acid γ/ml	Takata	Total serum protein %	Albumin/ globulin ratio	
	Males	Females						
Decom- pensated	Vascular heart diseases	1.19 ± 0.14 0.72 (26)	1.04 ± 0.21 0.43 (4)	5.9 ± 0.7 2.3 (12)	25.9 ± 1.8 5.9 (11)	1.3 ± 0.2 1.3 (29)	6.5 ± 0.1 0.8 (29)	1.4 ± 0.1 0.5 (13)
	Valvular heart diseases	1.69 ± 0.40 1.21 (9)	1.29 ± 0.26 0.83 (10)	5.2 ± 0.5 1.9 (17)	27.0 ± 2.2 8.2 (14)	2.2 ± 0.3 1.8 (27)	6.4 ± 0.2 0.9 (21)	2.1 ± 0.2 0.8 (10)
Compensated		0.64 ± 0.07 0.24 (17)	0.66 ± 0.08 0.21 (8)	5.4 ± 0.5 1.4 (7)	24.7 ± 2.0 5.0 (6)	0.9 ± 0.3 0.7 (6)	6.6 ± 0.2 0.7 (15)	1.8 ± 0.2 0.5 (7)
Normal		0.91 ± 0.04 a) (143)	0.76 ± 0.03 a) (141)	2 — 7 (10) b)	22 b) 17 — 27	0 — 1 c)	6.3 — 7.7 d)	1.3 — 2.2 d)

The values are $\bar{x} \pm \epsilon_{\bar{x}}$ mean $\pm$ error of mean
 s (n) standard deviation (number of cases)

a) acc. to Dahlberg & Josephson, (to be published).

b) acc. to Klin. Laborationsmetoder Astra, 1947.

c) acc. to Hafström, 1935.

d) acc. to Edsall J. T. (Clin. Lab. Diagnosis and Essentials of Hematology, Ann Arbor, 1948).

A numerical expression for the strength of the Takata reaction has been obtained in the usual way, by grading the precipitation in each tube according to strength by figures from 0 to 4. The sum total is used as a measure for the strength of the reaction. The means, obtained for Takata (see *table 27*), should be viewed with some reservation, as this reaction should above all be estimated individually and qualitatively. They, nevertheless, give some idea of the outcome of such a reaction, on a average. Generally opalescence is observed in 2—3 tubes, though flocculations occur, not infrequently, in some tubes. This stronger reaction may be seen more often in the group of valvular heart diseases. The results obtained are in fair agreement with HAFSTRÖM's (1935). He, further, observed no reaction or at most opalescence in one tube, in a normal material of medical students and nursing students.

In comparison with normal values, the observed values of total protein in congestive heart failure seem low, a fact that can probably be explained from the forementioned effect of dilution.

The albumin-globulin quotient conforms comparatively well to normal values.

No correlation has been observable between the *MCD* and bilirubin, citric acid, phosphatase, Takata, total protein and the albumin-globulin quotient, respectively.

Correlation between *MCD* and liver changes, based on autopsy cases of cardiac insufficiency.

At a selection from the autopsy material, from the point of view of homogeneity, an elimination has been performed of cases with hepatic changes suggesting a portal cirrhosis Laennec, with anamnestic data of hepatitis, when more than 7 months have elapsed from the determination of the *MCD* during the decompensation to the death of the patient. The thus obtained material has been divided according to the degree of severity into 3 groups, as follows:

- 1) cardiac cirrhosis.
- 2) nutmeg liver, excessive fatty infiltration.
- 3) other minor changes characteristic of stasis, such as accentuated contour.

Table 28. MCD in congestive heart failure with special regard to autopsy findings of liver.

		Cardiac cirrhosis	"Nut meg" liver	Mild forms of liver congestions
Vascular heart diseases	males	8.08 ± 0.14 0.24 (3)	8.08 ± 0.05 0.07 (2)	8.14 ± 0.11 0.25 (5)
	males	8.34 ± 0.38 0.53 (2)	7.86 ± 0.01 0.01 (2)	
Valvular heart diseases	females	8.20 ± 0.17 0.34 (4)	8.14 ± 0.02 0.03 (3)	7.80 (1)

The values are $\bar{x} \pm e\bar{x}$ mean ± error of mean
 s (n) standard deviation (number of cases)

In the *table 28*, the *MCD* values have been arranged with regard to type of cardiac affection, sex, and the degree of severity of the hepatic injury, respectively.

From the table a numerical tendency appears in the groups with valvular heart affections to higher *MCD* values at more severe hepatic injuries. On the other hand, in the vascular group, no such tendency is traceable. From the point of view of significance, no tendencies are, however, to be found. There should be justification for believing that the big variation in the *MCD* values cannot, principally, be explained by the hepatic changes, here observed.

III. SPECIAL INVESTIGATIONS REGARDING *MCD*

In some cases of cardiac insufficiency *MCD* has been determined simultaneously from arterial, venous and finger blood, 200 cells having been counted from each preparation. The results will be found in *table 29*.

In this limited material, no significant difference has been ascertained in the *MCD* of arterial, venous and finger blood, respectively. There is probably justification for stating that no real difference worth noting occurs.

Table 29. MCD in various blood samples as well as after oxygen inhalation.

Comparisons	Differences in <i>MCD</i>
between finger- and arterial blood	0.09 $\pm$ 0.08 (5)
between venous and arterial blood	0.007 $\pm$ 0.04 (6)
between finger- and venous blood	0.05 $\pm$ 0.05 (8)
of arterial blood before and after oxygen inhalation	-0.05 $\pm$ 0.05 (7)

$$\begin{array}{l} \text{The values are} \\ d \pm \varepsilon_d \quad \text{difference} \pm \text{error of difference} \\ (n) \quad \text{(number of cases)} \end{array}$$

No correlation has been traceable between the *MCD* and the O_2 - and CO_2 -content in the arterial blood, respectively.

Before and after an inhalation of oxygen (averagely 10 minutes), the *MCD* has been determined in arterial blood (7 cases). No change in the *MCD* has been observable in this connection.

No correlation between *MCD* and the amount of reticulocytes obtained on the same occasion has been ascertainable.

IV. WATER CONTENT OF THE ERYTHROCYTES

In accordance with the previously described P^{32} -method, the corpuscular mass is weighed after careful separation of the plasma through centrifugation and pipetting in a weighing-bottle with a cut-in stopper, and again, after drying in a thermostat at about 80° C. From this, the percentage loss in weight can be estimated.

CHAPTER XIII

Remarks on hemoglobin and corpuscular constants in cardiac insufficiency

In decompensated cases calculations have been performed of the corpuscular constants, mean corpuscular hemoglobin (*MCH*), mean corpuscular hemoglobin concentration (*MCHC*), and mean corpuscular volume (*MCV*) according to the ordinary formulae, as follows:

$$MCH = \frac{HB \times 15.0 \text{ g}}{\text{Red cell count}} \text{ } \gamma\gamma \quad \begin{array}{l} 15.0 \text{ g hemoglobin} = 100 \% \text{ } Hb \\ \text{acc. to Clinical Central Laboratory, Södersjukhuset.} \end{array}$$

$$MCHC = \frac{Hb \times 15.0 \text{ g}}{\text{hematocrit}} \%$$

$$MCV = \frac{\text{hematocrit}}{\text{Red cell count}} \text{ } c\mu.$$

The *MCH* has been estimated from finger blood only, while the *MCHC* has, as a rule, been derived from venous blood. At the calculation of *MCHC*, in certain instances the *Hb* factor in the dividend has been determined on finger blood. Correction was then made to a comparable value of venous blood with the previously stated factor of 1.04.

The material of decompensated cases comprises 34 men, including 26 with vascular, 7 with valvular heart diseases, one being pulmonic, and 15 women, 6 with vascular and 9 with valvular heart defects.

Table 31 contains the values recorded for decompensated men and women, together with the author's own normal values and normal values from the literature. As seen from the table the normal values for the corpuscular constants differ greatly among the investigators. In decompensated cases, however, lower values for *MCHC* and higher values for *MCV*, are noted as compared to the normal cases. For significance tests the author's normal cases have been used as comparison materials. Thus the difference between *MCHC* for decompensated men and normal men is seen to be almost significant ($t = 2.25^*$). The difference, however, between the *MCV* values for the same groups, is not proved to be significant. No significant difference has further appeared between *MCH* in decompensated cases and in normal cases.

Table 31. Corpuscular constants in congestive heart failure as well as in normal cases.

		$MCHC$ %	MCH γγ	MCV cμ
Decompensated	Males	29.7 ± 0.4 2.5 (34)	28.9 ± 0.2 1.1 (34)	97.7 ± 1.4 8.0 (34)
	Females	30.9 ± 1.0 4.0 (15)	28.0 ± 0.5 2.1 (15)	93.5 ± 3.1 11.8 (15)
Normal	Males	31.5 ± 0.7 3.3 (21)	29.1 ± 0.2 1.1 (21)	93.5 ± 2.3 10.3 (21)
$\bar{x} \pm \epsilon \bar{x}$ mean $\pm$ error of mean The values are s (n) stand. dev. (number of cases)				
Normal values	Males ^{a)}	34.8; 35.0; 32.4; 34.2; 33.4	30.0; 29.5; 31.4; 30.5; 30.9	85.9; 87.0; 96.5; 89.2; 92.6
	Females ^{b)}	32.5; 33.1	29.3; 28.8	90.3; 87.0

a) acc. to Wintrobe; Foster & Johnson; Walters; Andresen & Mugrage; Meyers & Eddy, resp.

b) acc. to Lewis, Ohlson, Cederquist & Donelson; Wintrobe, resp.

In the same material, consisting of 34 cases of decompensated men, the correlations between the total red cell volume and the $MCHC$ and the MCH , respectively, were studied. In this connection, corresponding values for $MCHC$ and MCH were observed in males with valvular and vascular heart affections. An inverted correlation was noted between the total red cell volume and the $MCHC$ ($r = -0.55 \pm 0.12$). It was highly significant ($t = 4.52^{***}$). The heterogeneity of the material has not contributed to this negative correlation, it has rather, as will be seen from fig. 21, increased the dispersion round the regression line, thus decreasing the value of the correlation coefficient.

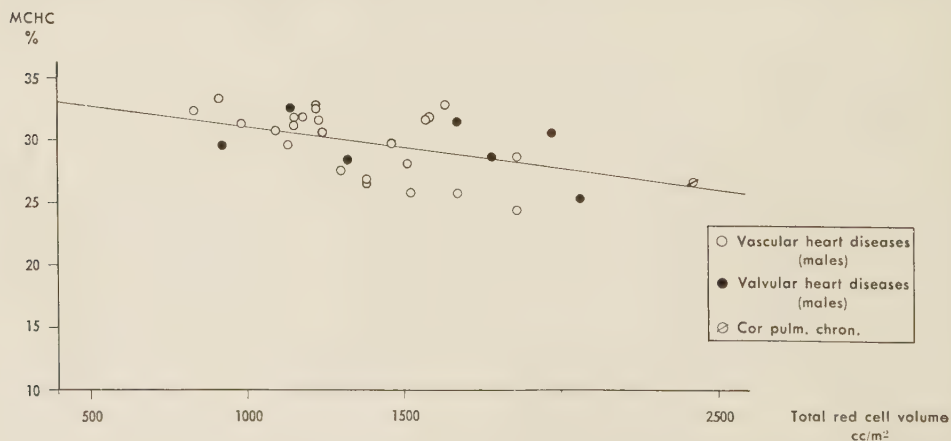


Fig. 21. Relationship between total red cell volume and $MCHC$ in congestive heart failure.

On the other hand, the correlation analysis performed on the same material has provided no support for the hypothesis of a correlation between total red cell volume and the *MCH* ($r = 0.20 \pm 0.16$; $t = 1.24$).

In a minor material of 14 decompensated men, in which both the *MCHC*, the *MCH* and the *MCV* had been determined from the same venous sample a correlation has been looked for between these three corpuscular constants. A highly significant inverse correlation was then noticed between the *MCV* and the *MCHC* ($r = -0.90 \pm 0.05$), while no correlation between *MCV* and *MCH* ($r = 0.34 \pm 0.24$, $t = 1.44$) could be ascertained. As will be seen from *Fig. 22 a* and *b*, the heterogeneity of the material has not contributed to the origination either of the significant correlation between the *MCV* and the *MCHC* or to the absence of any correlation between the *MCV* and the *MCH*.

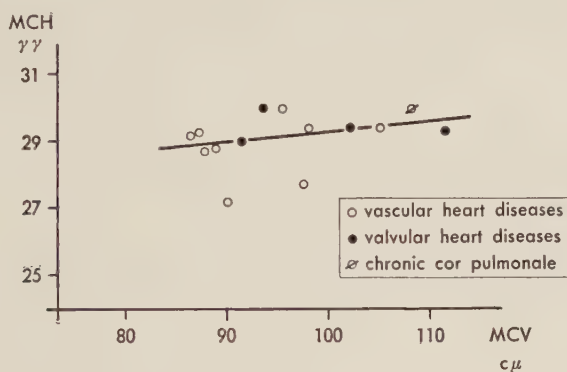


Fig. 22 a. Relationship between *MCV* and *MCH* of red blood corpuscles in congestive heart failure (males).

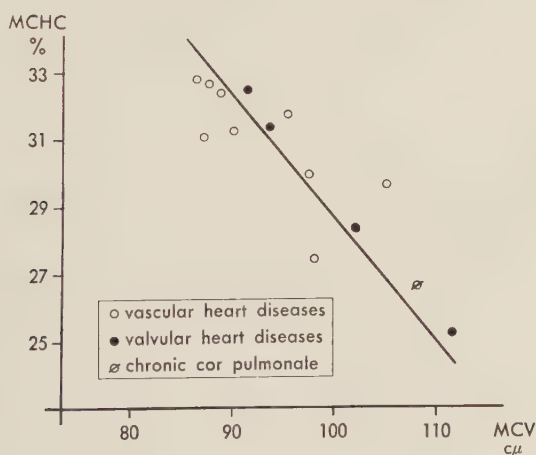


Fig. 22 b. Relationship between *MCV* and *MCHC* of red blood corpuscles in congestive heart failure.

No correlation has been noticed in this material of heart failures between total red cell volume and *Hb.*, *RBC* and hematocrit, respectively.

Analyses of men with vascular and valvular heart diseases disclose significant correlations between the *MCV* and the total red cell volume ($r = 0.66 \pm 0.11$, $n = 26$, $t = 6.00^{***}$ respectively $r = 0.66 \pm 0.21$, $n = 7$, $t = 3.11^*$).

On the other hand, no correlation has been ascertainable between these values for either females with vascular or females with valvular heart diseases or normal males.

When the observed correlation coefficients between *MCV* and total red cell volume as well as between *MCHC* and the latter are of another magnitude than the one observed between *MCV* and *MCHC* for decompensated males, and when the coefficients between *MCV* and total red cell volume for decompensated females and normal males not even could be estimated significant, it is unreasonable to state that the rise in the total red cell volume is simply to ascribe to an increasing volume of the individual blood corpuscles.

It may rather be supposed that the increase in the total red cell volume is above all due to an increased number of erythrocytes. However, a rise in *MCV* may have a part in an increased total red cell volume.

No correlation has been ascertainable between *MCD* and *MCV* in decompensated cases.

In 5 decompensated cases, determinations of the free erythrocyte protoporphyrin according to GRINSTEIN & WINTROBE have been performed. Throughout, values higher than normal were found.

The mean for these 5 cases is $111 \pm 13 \mu \text{ g}$, range 79–143 $\mu \text{ g}$, while the value for normal cases has been calculated at 31 $\mu \text{ g}/100 \text{ ml}$ of blood corpuscles, range 23–38 $\mu \text{ g}$ (CARTWRIGHT et al.).

Osmotic resistance of Red Blood Corpuscles in Congestive Heart Failure

Table 32. Osmotic resistance of the erythrocytes in congestive heart failure.

	Valvular heart diseases		Vascular heart diseases		Normal
	Males	Females	Males	Females	cases
Minimal resistance	0.493 ± 0.005 0.015 (8)	0.493 ± 0.007 0.029 (19)	0.492 ± 0.005 0.023 (24)	0.480 ± 0.004 0.012 (7)	0.42—0.48
Maximal resistance	0.308 ± 0.007 0.019 (8)	0.294 ± 0.007 0.031 (19)	0.298 ± 0.005 0.025 (24)	0.277 ± 0.015 0.039 (7)	0.32—0.36

It will be seen from the table that an initial hemolysis occurs at concentrations of sodium chloride that exceed the recorded normal values in the literature (0.42–0.48% *NaCl*), while a total hemolysis appears at lower concentrations (normal values 0.32–0.36% *NaCl*).

Age appears to have no influence on the osmotic resistance of the blood corpuscles for either group. Nor does the degree of insufficiency appear to be of any significance with regard to the osmotic resistance (see *table 33*).

8

Table 33. Osmotic resistance of red blood corpuscles in different stages of decompensation.
(men with vascular heart diseases)

	Minimal osmotic resistance ‰ NaCl	Maximal osmotic resistance ‰ NaCl
Insuff. group I	0.497 ± 0.006 0.024 (15)	0.300 ± 0.007 0.026 (15)
Insuff. groups II and III	0.484 ± 0.006 0.019 (9)	0.296 ± 0.008 0.024 (9)

The values are $\bar{x} \pm \epsilon \bar{x}$ mean $\pm$ error of mean
 s (n) stand. dev. (number of cases)

($r = -0.57 \pm 0.17$ $n = 15$ $t = 3.3^{**}$) while for women, in this respect, a tendency, but no significant correlation, has been observable ($r = -0.47 \pm 0.28$ $n = 8$ $t = 1.7$). This absence of statistical significance in the latter correlation may well be explained by the limited material available for the calculation ($n = 8$).

On the other hand, no correlation between the *MCD* and the osmotic resistance can be traced for either men or women in cases belonging to the insufficiency group I, where the degree of edema is most pronounced. The correlation coefficient is for men as follows: *MCD* and initial hemolysis ($r = -0.11 \pm 0.25$; $n = 16$), *MCD* and total hemolysis ($r = 0.37 \pm 0.22$ $n = 16$); and for women: *MCD* and initial hemolysis ($r = 0.11 \pm 0.25$ $n = 16$), *MCD* and total hemolysis ($r = 0.00$ $n = 16$).

A Z-test discloses an almost significant difference between the correlation coefficients for the *MCD* and total hemolysis for men in the insufficiency groups II and III and insufficiency group I ($t = 2.57^{*}$), while, on the other hand, no statistically significant difference is ascertainable between the correlation coefficients for the *MCD* and initial hemolysis in the corresponding insufficiency groups.

Osmotic resistance during treatment.

The osmotic resistance of the blood corpuscles has been observed during the treatment of the cardiac insufficiencies in 43 cases, belonging to the insufficiency groups I and II, regarding which it is known that a considerable change in the clinical condition may occur when brought to compensation. No classification has been performed with regard to sex in this material, since there are no indications in results arrived at of any differences in the normal values between men and women, as far as osmotic resistance is concerned, and these normal values should be reached, when cases of cardiac insufficiency are brought to practically complete compensation. 32 of the 43 cases have been considerably improved, clinical compensation being reached or almost reached. In the other 11 cases only a slight improvement has been noted during the observation time, or none at all.

The values of the osmotic resistance for each individual have been arranged in a chronologic order, stated in weeks from the first determination, minimal and maximal

resistance separately. With regard to periods where observations are lacking, the resistance has been estimated through linear interpolations between adjacent observed values. On the basis of the observed and "theoretical" values from all the individuals, an average value has been fixed for every week. Then, a straight line has been drawn according to the thus obtained weekly means. It has been made to describe the course of development.

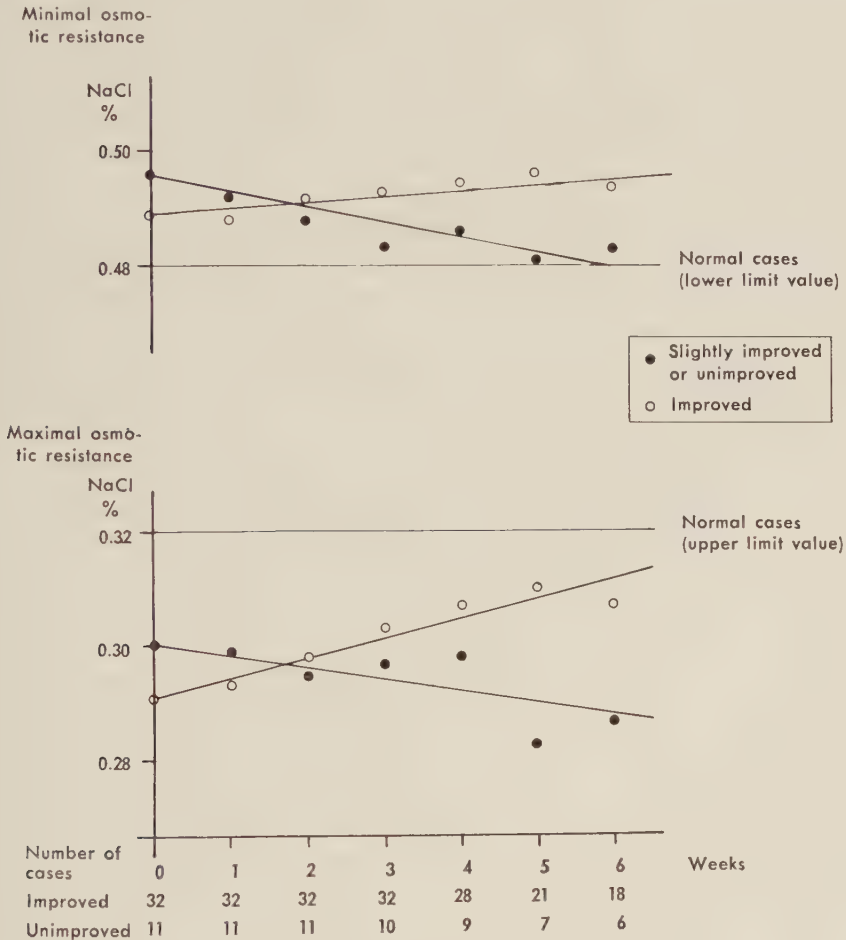


Fig. 23. Osmotic resistance of erythrocytes in congestive heart failure during treatment.

Fig. 23 illustrates the changes in the osmotic resistance with the time for the two groups. In fig. 23 lines have also been inserted showing normal values for maximal and minimal resistance in a normal material. In the group of clinically compensated cases, the maximal resistance is observed to decrease from an elevated value during the

decompensation, approaching normal values. The minimal resistance from the same group is all the time reduced, being, on the whole, constant during the observation period.

In cases, on the other hand, where the condition of decompensation does not show any evident change, the curves of resistance run differently. The maximal resistance increases during the observation time, revealing during the entire course an elevated value, as compared to a normal material. The minimal resistance increases during the same period from values that show a reduced resistance in relation to the normal values.

For each individual, differences have been calculated between the first and last observed values of maximal and minimal resistance. In the compensated group, the mean difference in maximal resistance is found to diverge significantly from zero, while this does not happen with regard to minimal resistance. Yet, in both instances, an average decrease is notable.

In the group of clinically but slightly improved cases, significant differences are obtained between the means in the minimal, but not in the maximal resistance. Here, the differences, however, indicate an increase in resistance (see *table 34*).

Table 34. Changes in osmotic resistance of erythrocytes during treatment of congestive heart failure.

	Improved	Slightly improved or unimproved
Mean difference of minimal resistance	-0.004 ± 0.004 1.0 (32)	0.015 ± 0.006 2.5* (11)
Mean difference of maximal resistance	-0.018 ± 0.006 3.0** (32)	0.011 ± 0.007 1.6 (11)

$$\begin{array}{l} \text{The values are} \\ d \pm \varepsilon_d \quad \text{difference} \pm \text{error of difference} \\ = \\ t \quad (n) \quad t = \frac{d}{\varepsilon_d} \text{ (number of cases)} \end{array}$$

It should be noted that the class interval is big, as compared to the observed differences. This causes a deficiency in the prerequisites for a *t*-analysis, and, accordingly, it becomes more difficult to ascertain significant differences. It is not impossible, therefore, that the not statistically significant mean difference might have been denoted as actually significant.

CHAPTER XV

Serum Iron in Congestive Heart Failure

The material on which the present investigations of serum iron have been performed, as well as the methods applied, have been accounted for earlier. The means for serum iron in decompensated men and women with vascular and valvular heart affections are to be found in *table 35*. There, the normal values found by VAHLQVIST (1941), and those collected by him from other authors, have been tabulated. It should be noted that VAHLQVIST's own normal values keep significantly higher than those of other authors. Accordingly, when comparing the values from the various groups of cardiac insufficiencies with those from the normal materials, a too high value should not be attributed to the statistical evidence.

Table 35. Serum iron (mg %) in congestive heart failure.

	Vascular heart diseases	Valvular heart diseases	Normal cases acc. to Vahlquist (1941)	Normal cases (acc. to other authors*)
Males	0.074 ± 0.009 0.044 (22)	0.125 ± 0.012 0.035 (8)	0.142 ± 0.006 0.043 (50)	0.122 ± 0.002 0.023 (110)
Females	0.051 ± 0.011 0.026 (6)	0.076 ± 0.015 0.049 (11)	0.123 ± 0.005 0.032 (50)	0.104 ± 0.003 0.026 (110)
Values corrected for dilution:				
Males	0.096 ± 0.012	0.148 ± 0.015		
Females	0.064 ± 0.014	0.095 ± 0.019		

*) Heilmeyer & Plötner (1937), Moore et al. (1937), Skouge (1939), Bröckner-Mortensen & Olsen (1940), (from Vahlquist 1941).

$$\begin{array}{l} \bar{x} \pm \epsilon_{\bar{x}} \quad \text{mean} \pm \text{error of mean} \\ \text{The values are} \quad = \\ s \quad (n) \quad \text{standard deviation (number of cases)} \end{array}$$

From the table, the serum values for both men and women with vascular heart affections are seen to be significantly lower than those of the two normal groups.

For women with valvular heart affections, the values are numerically lower than the serum iron values of the two normal groups. Still, it is only when comparisons are made with VAHLQVIST's own normal values that a significant difference appears ($t = 2.94^{**}$

$n = 61$). As far as the valvular men are concerned, a lower value has been recorded than those of the two normal values, though without any significant difference.

It should, however, be kept in mind that the serum iron values are relative, calculated on a certain voluminal unit of plasma, and that lower values are to be expected on account of the dilution of the content of the plasma brought about by the decompensation. Proceeding from the observed increases in the plasma volumes of men and women with cardiac insufficiency, correction factors have been worked out. By means of these factors, which as a matter of course are but approximate, the recorded serum iron values are corrected. The actual correction is of course done on the plasma volume, but, in practice, this comes to the same as correcting the relative serum values. The correction factors are for men and women $\frac{1}{1.29}$ and $\frac{1}{1.25}$ for the plasma volume and 1.29 and 1.25, respectively, for the serum iron.

The thus corrected values for the serum iron in the material of cardiac insufficiencies can be seen in the table. At a new comparison with the normal materials, the values for both men and women with vascular heart affections, are found still to be numerically lower. However, it is only from VAHLQVIST's own normal material that the differences are considerable from the point of view of statistical significance. As far as the valvular groups are concerned, the value for men looks rather high, while, for women, lower than the two normal ones without any significant difference.

When comparing the various groups of heart affections, paying regard to the sex factor, the following findings are made, no matter whether the values applied be corrected or not.

Numerically lower values in the vascular than the corresponding valvular groups and lower values for women than for men are obtained. When tested by t -analysis, the difference between vascular and valvular men ($t = 2.23$, ** $n = 30$) and that between valvular men and women are alone significant ($t = 2.58$ ** $n = 19$).

Consequently, a tendency to lower values for serum iron may appear in vascular, as compared to valvular heart affections, as well as to lower values for women than for men. In this instance, the age factor may have made itself felt, seeing that, averagely speaking, the living age of vascular men and women is higher than that in valvular heart affections. Thus, DAHLBERG and JOSEPHSON have observed significantly lower values for serum iron in elderly individuals (70—80 years of age) than for those below 50 years of both sexes.

It should be noted, however, that the dilution effect may have varied from one group to another and that this may have affected the observed values.

When studying the values of serum iron and total red cell volume in the group of men with valvular heart affections higher values of the red cell volume than normal and rather high serum iron values will be observed, while in the group of men with vascular heart diseases the former were higher than normal but the latter lower. Similar studies in the female groups disclose higher values of the cell volume and lower values of serum iron than normal.

Therefore it is not surprising to find that in the male group of valvular heart affections a relatively marked and significant correlation occurs between serum iron and total red cell volume ($r = 0.74 \pm 0.18$; $n = 6$; $t = 4.0^{**}$).

This applies equally to the lack of more marked correlations between the factors for the other groups (males with vascular heart affections: $r = 0.34 \pm 0.20$, $n = 19$, $t = 1.67$; females with vascular heart diseases: $r = 0.36 \pm 0.36$, $n = 6$; and females with valvular heart defects: $r = 0.29 \pm 0.37$, $n = 6$).

In cases where compensation has been achieved through the treatment, the correlation between the content of serum iron and the osmotic resistance in the blood corpuscles has been tested. At the time of the compensation, the recorded values for the serum iron are found to be correlated to the maximal resistance of the blood corpuscles, the more reduced the resistance, the higher the serum iron (the correlation coefficient being 0.74 ± 0.13 $n = 11$ $t = 5.5^{***}$). Considering that men with vascular heart affections predominate in the material, while the other groups are represented only by a few individuals, calculations have been performed exclusively on the first-mentioned group. No correlation is traceable between serum iron and initial hemolysis, however, as just mentioned, the latter is reduced throughout.

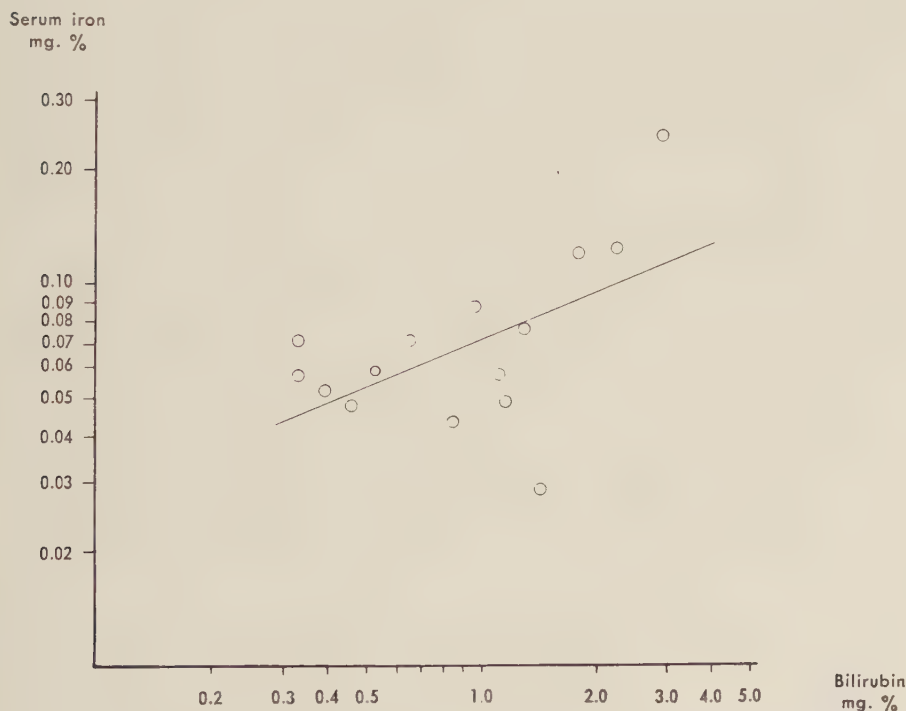


Fig. 24. Relationship between bilirubin and serum iron in decompensated vascular heart diseases (males).

An investigation of the correlation between serum iron and bilirubin in decompensated cases has disclosed a highly significant correlation in the group of men with vascular heart affections. ($r = 0.80 \pm 0.11$; $n = 10$; $t = 7.28^{***}$) In other groups, no significant correlations have been found. However, these groups only contain a few cases. (Fig. 24).

Serum iron during treatment.

The serum iron values were followed in the treatment of heart failure in 28 cases belonging to insufficiency groups I and II. A considerable change in the clinical course of these cases is known to take place provided they can be brought to compensation. 20 of these cases improved markedly with consequent compensation, almost or entirely. In the remaining 8 cases, only a slight improvement was obtained during the observation period, or none at all.

The values of serum iron for each individual have been arranged in order of time, in weeks from the first determination. Whenever observations have been lacking, values have, as previously stated, been fixed by means of linear interpolation between adjacent observed values. From the observed and "theoretical" values from all the individuals, an average value has been obtained for each week. Then, a straight line has been drawn adapted to the thus calculated weekly means, to represent the course of development.

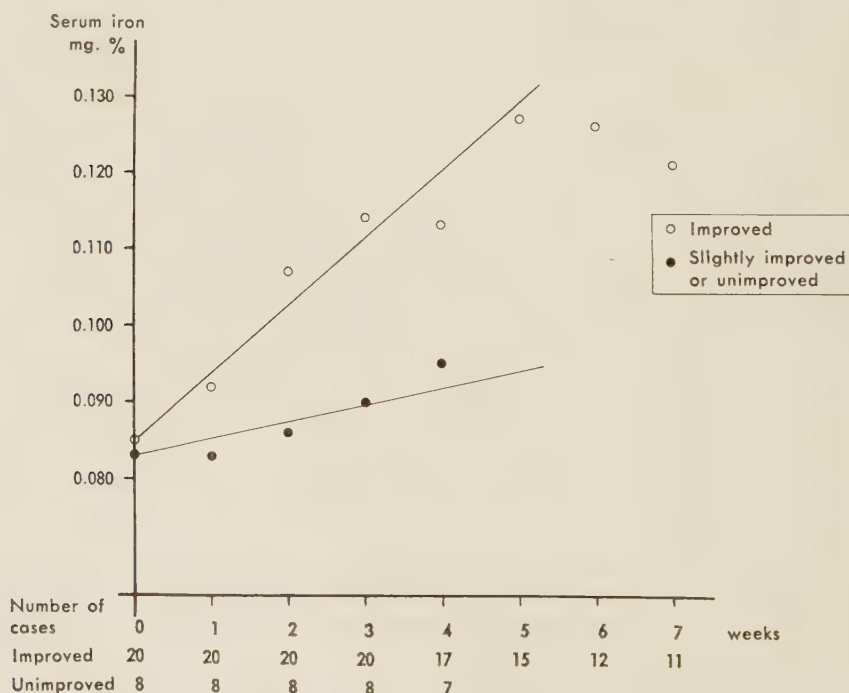


Fig. 25. Serum iron (mg %) in congestive heart failure during treatment.

CHAPTER XVI

Discussion of the results

CHANGES IN BONE MARROW

From the present investigations, an activation of the erythropoiesis seems to take place in the examined material of cardiac insufficiency. The number of nucleated red blood corpuscles exceeds, those recorded in most normal materials. The noted mean of the number of nucleated red blood corpuscles in the bone marrow from sternum corresponds with that observed by OTT in decompensated heart affections. In the present material, the values of valvular and vascular heart affections conform well.

In the survey of the literature, the normoblasts were stated to continue to divide mitotically, until finally a stage is reached when this ceases and the nucleus undergoes a pycnotic degeneration. Simultaneously, the basophilie becomes less and less demonstrable, while the cytoplasm obtains its full complement of hemoglobin. Obviously, therefore, the passing from one stage to another of the erythropoiesis is indefinite. Viewed in this light, it may be a question of definition whether the erythrocytes emanate from normoblasts, to be designated as orthochromatic, or as polychromatic. Yet, to conclude from the literature, a certain small number of orthochromatic normoblasts have, it seems, degenerated to the point of losing their capacity to regenerate erythrocytes. As far as the hemoglobin synthesis is concerned, THORELL (1948), among others, has shown that, during a development from polychromatic to orthochromatic erythroblasts, the hemoglobin concentration (*MCHC*) increases, on account of the volume decrease, the amount of hemoglobin (*MCH*) remaining relatively unchanged.

In the present material of cardiac insufficiency, the orthochromatic erythroblasts reveal a value comparatively exceeding that of the normal, while, at the same time, the mitotic number remains high, this may suggest that an intensified formation of such cells has taken place. Also, an increase of the more mature erythroblasts has been observed in cardiac insufficiency (by OTT and BRAUN), as well as (by ZADEK) in polycythemia at high altitudes.

It further appears from the investigations that the *MCHC* of the erythrocytes in the peripheral blood is correlated to the quotient of orthochromatic/polychromatic erythroblasts in the bone marrow, and that the *MCD* of the erythrocytes discloses a correlation to the quotient between percentage numbers of macroforms of these two erythroblastic cells. In the peripheral blood, also polychromasia is noted. Thus, not unlikely, in the

course of the exaggerated erythropoiesis that is observed in cardiac insufficiency, the erythrocytes may originate both from the orthochromatic and the polychromatic erythroblasts. Anyhow these observations may indicate that the bone marrow function has been disturbed.

On the basis of what has been stated regarding the hemoglobin synthesis, it is, further, but reasonable that no correlation has been ascertainable between the forementioned quotient and the *MCH* of the red corpuscles in the peripheral blood. In this connection, it should be noted that no correlation has been traced between the *MCD* of the polychromatic erythroblasts and the corresponding values of the erythrocytes in the peripheral blood.

A study of the distribution of the mitotic numbers among different erythroblastic cells disclosed that, in vascular cardiac insufficiency, relatively high values were to be found in the basophilic erythroblasts, successively diminishing in the older forms of erythroblasts. In valvular cardiac insufficiency, on the other hand, comparatively higher values were noted in the polychromatic and orthochromatic erythroblasts. It should be mentioned that, in the erythroblastic system of normal bone marrow, 91 per cent of the dividing cells were late, 9 per cent being early erythroblasts (JAPA 1943). Apparently, a deviation of the mitotic maximum occurred to younger forms of erythroblasts, particularly in the vascular heart affections. WILTON (1938) termed this a left deviation of the zone of growth. This may indicate that the erythropoiesis should be to a more maximal degree engaged in the vascular heart affections. However, the possibility cannot be excluded that the deviation of the percentage mitotic figures in the group of vascular heart diseases, as compared to the valvular group, can be attributed to the relatively lower serum iron values observed in the former.

In the material of cardiac insufficiency, the occurrence of macroblasts has also been noted. They appear to be differently distributed among different erythroblastic cells. Also, the types of distribution can be seen to differ from vascular to valvular heart affections. It is difficult to say what this may signify. Different populations of macro- and normoforms, respectively may divide themselves differently in different erythroblastic stages. The question whether a relation is to be found to the strength of the cell production has also to be left open. To answer this question presupposes special studies.

Certain problems connected with the findings in the bone marrow will be discussed in later chapters.

STIMULATION OF ERYTHROPOIESIS

The results of the present investigation indicate that in congestive heart failure a stimulation of erythropoiesis takes place. Not unlikely, the explanation is ultimately to be found in the hypoxia that occurs in decompensation. Thus, both the number of erythroblasts in the bone marrow and the reticulocyte figure are correlated to the arterial oxygen saturation. The reduced arterial oxygen saturation is one fundamental

cause of the low tissue oxygen tension, contributing factors being the slowing of the blood flow and the increased metabolic rate found in heart failure.

Whether the anoxia exerts a direct stimulating effect on the erythropoiesis, or whether it operates through hormones produced by some organs, remains an open question. There is much indicating that endocrine organs interfere. Hypoxia has been shown not to bring any stimulating effect to bear on the bone marrow, when a hypophysectomy has been performed (MEYER, STEWART, THEWLIS & RUSCH, 1937), while at thyroidectomy the hypoxia elicits such an effect. It is also known that a protracted *ACTH*-treatment will give an increased total red cell volume, at the same time as a hypertrophy of the adrenal cortex is being observed. In animals in low-pressure chambers, an increase in the weight of the adrenals was observed, while no established variations in the weight of the thyroid and the hypophysis were ascertainable (SUNDSTROEM & MICHAELIS, 1942). In some cardio-vascular lesions a hyperactivity of the adrenals has been noted (SELYE).

It seems likely that account has to be taken of an influence on the function of the bone marrow from a local hypoxia, at the same time as the hypoxia, perhaps via some endocrine organs elicits a general adaptation in the various organic systems of which the erythron must be regarded as one.

Accordingly, in this or that manner, the hypoxia brings on the stimulation of the bone marrow that, *inter alia*, reveals itself in an increased number of nucleated red blood corpuscles in the bone marrow and a reticulocytosis.

TOTAL RED CELL VOLUME

It has emerged from these investigations that decompensated heart affections show a bigger total red cell volume than a control group. It has also been noted that in this material of cardiac insufficiency, the morbid condition obviously affects the dispersion more than the mean. The reason may be that the total red cell volume in some individuals has markedly increased, while, in others, it has remained practically normal.

A study of the conditions for this increase in the total red cell volume suggest that it may take place, in a different way in vascular and valvular heart affections. In the vascular group, an increased total red cell volume occurs early. It does not seem to grow notably with the duration of an insufficiency. In the valvular affections, on the other hand, a rising total red cell volume is noted with the progress of the cardiac insufficiency, from relatively normal up to considerably elevated values.

From the bone marrow investigations, the vascular heart affections seem to have higher mitotic values in younger erythoblasts than the valvular. Since the stimulation that elicits the erythropoietic increase is likely to be identical in both these conditions of cardiac insufficiency, i.e. the anoxia, the vascular heart affections may be supposed already to have passed the stage in which the valvular affections are to be found when subjected to examination for their decompensation. This seems to explain why higher values for the total red cell volume are early to be noted for the vascular than for the valvular affections, while the latter increase their total red cell volume with the duration of the insufficiency. Since the erythropoiesis seems to be to a more maximal degree

engaged in the vascular heart affections, its reserve capacity is, supposedly, relatively diminished. Nor has, as previously mentioned, any noteworthy increase in the corpuscular amounts been observable with a prolonged duration of an insufficiency. However the possibility cannot be excluded that the relatively lower serum iron values observed in cases with vascular heart diseases could limit the capacity for red blood cell regeneration, provided these values signify an iron deficit, the demand for available iron being exaggerated in increased cell production.

To judge from the results, it is, further, to be presumed that the vascular heart affections increase their total red cell volume with the degree of decompensation. For benign forms of decompensation, no significant increase in total red cell volume has been recorded, while definite increase is noted in the graver cases of decompensation. When further inquiring into the influence of the symptoms that condition a decompensation, the same tendency to increase in the total red cell volume with the degree of severity of the edema is to be noticed. No correlation between venous pressure and total red cell volume has been traced.

As far as the valvular heart affections are concerned, no rise in the total red cell volume has been ascertainable either with the degree of insufficiency or edema or with the venous pressure.

The differences in behaviour between vascular and valvular heart affections, with regard to the variations in the total red cell volume, disclose a further relevance when examining more closely the clinical development in these two groups of heart affections.

The characteristics of the valvular rheumatic heart affections, here concerned, are from year to year gradually proceeding, slow deterioration in the cardiac function, interposed by periods of decompensation of more or less short duration, with a tendency to occur more frequently in the course of the development. Consequently, a patient can for a long time be in a clinically satisfactory condition, in spite of a continued deterioration in the function of the heart, to judge, e.g., from heart function tests. As long as only latent symptoms of cardiac insufficiency are to be noted, values within normal limits for arterial oxygen saturation and cardiac output have generally been recorded. When symptoms of insufficiency appear at rest have decreasing values for cardiac output and arterial oxygen saturation been observed (ELIASCH 1952).

From a clinical point of view, it is therefore acceptable that the total red cell volume increases with the duration of the manifest symptoms of insufficiency.

Once a decompensation appears in cases with cardiovascular affections, it appears to develop more violently and to lead to a lethal issue more rapidly. At an examination, varying degrees of decompensation may, of course, be met with. It is, on the other hand, possible that these patients in many instances have, before an acute decompensation has set in, suffered from an hypoxia of a relatively low degree.

The mean age in this group of heart affections is higher than in the valvular group. It should, accordingly, be borne in mind that there is, with higher age, a tendency to a decrease in the cardiac output through a diminished oxygen consumption (LEWIS 1938), as well as a diminution in the arterial oxygen saturation (DILL, GRAYBIEL, HURTADO,

TAQUINI 1940), a fact ascribed to a lack of uniformity in the ventilation of the lung lobes. These factors produce a decrease in the oxygen tension in the tissue.

There are, therefore, reasons to suppose that old patients with cardiovascular affections, but without any cardiac decompensation, may be able to live fairly long with an hypoxia. This should result in a progressive bone marrow stimulation. At an examination during decompensation a relatively big total red cell volume that will only slightly increase with the duration of insufficiency should be expected, as well as, in the bone marrow, higher mitotic values in the younger erythroblasts. It should, of course, be noted that, in this connection, the bone marrow should have retained its capacity to respond to stimulation. To conclude from investigations by SEGEL & REIHER (1939), among others, the influence of age on the bone marrow apparently implies a possibility of a more marked fatigability at increased stimulation in cases with a higher age, even though the sternal punctates fail to reveal any numerical changes in comparison to younger individuals (SEGERDAHL 1935 PLUM, 1941). The influence of an iron deficit has already been suggested.

In the present material of cardiac insufficiency, a possibility of compensation in the bone marrow may have occurred in elder patients, since no correlation between age and total red cell volume has been ascertainable in the various groups of cardiac insufficiencies.

It further appeared from the results that the valvular groups disclosed higher numerical values of the total red cell volume than the corresponding vascular insufficiencies. The differences could, however, not be proved to be significant. From the investigations discussed above, of the conditions determining the increase in blood corpuscles in valvular and vascular heart affections, it follows that the result of a comparison between the different groups of heart affections is dependent on various factors influencing the total red cell volume, such as the duration of congestion, the degree of decompensation and, possibly, the serum iron content.

It is, therefore, only to be expected that other investigators will arrive at other results from such comparisons particularly if they have observed a more limited material, not sufficiently representative in the last-mentioned respect. Thus, PRENTICE, BERLIN, HYDE, PARSONS, LAWRENCE & PORT (1951) had noted increased total red cell volume in decompensated rheumatic valvular defects, while in hypertensive and arteriosclerotic heart diseases only slightly increased or normal values were noted.

In the control groups, the men were seen to have a higher total red cell volume than the women. This conforms to the results of other authors (BERLIN and others). The same tendency appears from the present material of cardiac insufficiencies.

PATHOGENESIS OF THE MACROCYTOSIS

In Chapter VIII, regarding bone marrow findings in cardiac insufficiency, the presence of macroblasts in the bone marrow was pointed out. Also in the peripheral blood a macrocytosis has been observed in cardiac decompensation, as manifested by an increase in the *MCD*.

The occurrence of polychromasia in the peripheral blood, as well as the ascertainment of a correlation between the *MCHC* in the forementioned erythrocytes and the quotient of orthochromatic/polychromatic erythroblasts in the bone marrow, have lent support to the assumption that the erythrocytes in the peripheral blood may originate from different forms of erythroblasts. Furthermore, a correlation has been shown to exist between the quotient of the percentage number of macroforms of orthochromatic erythroblasts/the percentage number of macroforms of polychromatic erythroblasts and the *MCD* of the erythrocytes. It does not seem improbable, therefore, that anisocytosis in the peripheral blood is conditioned by different populations of erythrocytes, emanating from different erythroblastic cells, dissimilar both in maturation and size.

It is, further, likely that the recorded Price-Jones curves of the erythrocytes during decompensation consist of different populations of red blood corpuscles, differing in mean diameters. By means of special methods of analysis, LARSEN and MOGENSEN claimed to be able to show that, in hepatic diseases, at least two populations with different mean diameters are to be found.

As no analytic methods of a general applicability have not yet been elaborated, which can be considered quite reliable, from a statistical point of view, the present author has refrained from any attempt to deduce subpopulations.

Very likely, therefore, the macrocytosis in the peripheral blood has a central origin. The question remains whether, in cardiac insufficiencies, also peripheral factors may influence the form of the erythrocytes. No definite differences in the diameter of the erythrocytes in arterial, venous and finger-blood have been traceable. Nor has any change in the *MCD*, after a short inhalation of oxygen, been notable, nor any correlation between the *MCD* and the oxygen or carbon dioxide content in the arterial blood.

These results conform well to those of the forementioned investigations by WARBURG, VAN SLYKE, WU & MACLEAN and DRYERRE, MILLAR & PONDER.

There remains the osmotic influence, if any. It should exert a swelling effect on the erythrocyte, increasing its thickness, while diminishing its diameter. Without such a supposed influence, the measured *MCD* should, accordingly, be bigger. Such an occurrence derives support from the ascertained increase in the fluid content of the blood corpuscles with the degree of a cardiac insufficiency. This could be explained from an accumulation of osmotically active products in the cells. Such a product is to be found in lactic acid, the formation of which has been shown to happen more rapidly in the blood corpuscles than in the plasma during a stasis (TSAI CHIAO, CHEN & CHIU).

On the other hand, younger blood corpuscles that are formed during regeneration (ODA, 1927) have a lower specific gravity than mature erythrocytes. The increased fluid content in the erythrocytes during a decompensation can, therefore, at least partly, be explained by the appearance of young cells. The present investigations lend strength to such an assumption. LARSEN (1951) has likewise noted that the macrocytes that occur in hepatitis show a fluid content bigger than normal. It is, however, not unlikely that both these interpretations can be correct.

What, then, is the genesis of a macrocytic normoblastic erythropoiesis? This question cannot be definitely answered but certain points of view can be set forth. In animal experiments (PLUM & HEDLUND 1952), a partial stenosis was applied to the vena cava inferior below the junction of the venae hepaticae. In these experiments, no evident changes in the structure of the liver could be detected. On the other hand, edemas were found in the thigh and abdominal wall as well as ascites. A certain correlation could be observed between the arterial oxygen saturation and the *MCD* of the polychromatic erythroblasts in the femoral marrow, although the minimum of oxygen saturation precedes the maximum of the *MCD* in time. Since *MCD* of the polychromatic erythroblasts in the humeral marrow remain unchanged, no general influence on the bone marrow is likely to be attributed to the anoxia. The anoxia may act as an incitement in the area of the stasis, but there the venous stasis seems to constitute the most essential factor in the origination of a macrocytosis. Similarly, it could be shown that, with a decreasing stasis, probably on account of a development of collaterals, the *MCD* of the polychromatic erythroblasts in the femoral marrow will again decrease, while at the same time the arterial oxygen saturation increases.

In the present clinical material of cases of cardiac insufficiency, no correlation between the arterial oxygen saturation and the *MCD* of the polychromatic erythroblasts in the sternal marrow or the erythrocytes in the peripheral blood has been ascertainable. Considering the observations in the animal experiments viz, that a deviation in time occurs between the minimum in arterial oxygen saturation and the maximum of the *MCD* in the corresponding erythroblasts, the fact that no correlation has been found in that instance, in the present clinical material, seems explicable.

On the other hand, in the groups with the gravest symptoms of decompensation (groups I and II), significantly elevated *MCD* values will be found for both types of heart affections, while the most benign cases (group III) show values that conform well to those of the normal materials. Analogous observations have been made with regard to the *MCD* values in the different edematous groups of both vascular and valvular heart affections. No correlation has, on the other hand, been traced between the *MCD* values and the venous pressure.

It, likewise, emerges from the ascertained correlation between the duration of insufficiency and the *MCD* in the vascular heart affections, that the development of a cardiac decompensation to some extent affects the size of the *MCD* in the erythrocytes. As far as the valvular heart affections are concerned, no statistically significant correlation has been found between these two variables.

As a hypothesis, the actual difference that is noted between vascular and valvular heart affections, with regard to the correlation between the *MCD* of the erythrocytes and the duration of an insufficiency, may, possibly, be attributed to the difference in clinical progress between the two types of affections, as previously described.

The macrocytosis that has been observed in hepatic diseases has, inter alia, been attributed to the actual dysfunction of the liver. It is, for this reason, relevant to discuss

the part that such an impairment may play in the origination of a macrocytosis in cardiac insufficiency.

From the described autopsy material, the *MCD* seemed unaffected by the degree of a hepatic injury. Supposing that such an injury constituted the decisive factor, an increasing macrocytosis might be expected with an enhanced destruction of the liver parenchyma. On the other hand, BOLAND and WILLINS have been unable to ascertain any difference in the liver function in cases of cardiac cirrhosis and liver congestion. The liver dysfunction that is to be noted, irrespectively of any liver destruction, might nevertheless, be supposed to affect the origination of a macrocytic erythropoiesis. Among the liver tests that have been performed here, the bilirubin, and Takata show that a disturbance in the liver function occurs. Still, no correlation between these tests and the *MCD* in the erythrocytes has been recordable. The diminution in the *MCD*, noticed with the decrease in the liver, in cases that have reached compensation, may, in fact, be two parallel effects of a diminished stasis.

From these points of view, based on a material of cardiac insufficiency, it may be worth while to discuss further the causes of a macrocytic erythropoiesis in a hepatic cirrhosis. Such an analysis is justified also because of the fact that, in a portal cirrhosis, an elevated venous pressure in the abdomen and an ascites, as well as a leg-edema, are often to be noted. In addition, an increase in the plasma volume has been recorded (PERERA 1946, HILLER, HUFFMAN and LEVEY 1949, BATEMAN, SHORR and ELGVIN 1949). This increase has been shown by PERERA to be correlated to a portal hypertension. KEYS and SNELL (1938) have, likewise, found a reduced arterial oxygen saturation in liver cirrhosis and other conditions with severe parenchymatous liver injuries. KEYS and SNELL attribute the anoxia to some abnormality in the blood or a change in the ventilation of the lungs, since the possibility of an edema in the alveolar wall cannot be excluded.

It is of interest to note that REIN (1942) observed a myocardial insufficiency, secondary to a hepatic injury, and that OPPENHEIM (1950) stated that a myocardosis often is found in a primary liver cirrhosis. He regarded it as a hepatogenic metabolic disease in the heart muscle, characterized by electrocardiographic changes, dyspnea and cyanosis, together with hypoproteinemia.

Clinical data, such as the degree of an edema and an ascites, have been submitted by MEULENGRACHT and GORMSEN (1948), in their examinations in cases of subacute and chronic atrophy of the liver. An analysis of their material will show a mean for the *MCD*, in the twelve cases with ascites and edema, of 8.25 ± 0.09 , while in twelve other cases without any symptoms of stasis the mean of the *MCD* amounted to 7.82 ± 0.06 . The difference is highly significant ($t = 3.9^{***}$). All cases were women.

In this connection, mention may again be made of the fact that LARSEN has noted a decrease in the *MCD* in same cases of acute hepatitis after treatment with niacin. Niacin is, however, also an effective agent for dilating vessels, used in cardiologic practice. This is of particular relevance when, in conditions of severe parenchymatous hepatic injury, an arterial anoxemia has been noted. (KEYS & SNELL).

When, as shown in the present investigations of cardiac insufficiency, the erythropoiesis is normoblastic, it is, consequently, unlikely that, in cardiac insufficiency, disturbances should occur in the formation of the hemopoietic principle, as in the case of pernicious anemia and other perniciosiform anemias. On the contrary, it has been possible to show that preparations made from liver of cases with cardiac insufficiency have caused a remission in cases with a pernicious anemia (SHIFF and others). Accordingly, there is no reason to expect that a liver therapy should exert any influence on this disturbance in the erythropoiesis in cardiac insufficiency.

An observation further worth noting is that recorded by GOLDBERG, HAMMARSTEN, HELLSTRÖM, LINDGREN & PLUM (1950), viz, that a macrocytosis that appears after a ligation of the common bile duct is less severe when the animal operated upon has been treated with *ACTH* or splenic extract.

In sum, it seems, probable that a macrocytosis, originating from a macro-normoblastic erythropoiesis in cardiac insufficiency, has a causative relation to the venous stasis, whether through the tissue hypoxia connected with it or in combination with some endocrine organs. Investigations dealing with these problems are in progress. (Plum & Hedlund).

ON OSMOTIC RESISTANCE OF ERYTHROCYTES

From the present investigation it emerges that a reduced minimal and an elevated maximal resistance are to be noted in decompensated cardiac affections. The results conform to those of FRANCESCON, MELDOLESI, BUCCIANI and ROSSI. A reduced minimal resistance has also been observed by WALLER, BLUMGART & VOLK. No definite influence of the type or degree of a cardiac affection, or of the age of a patient has been ascertainable. The lastmentioned result conforms to those of WIECZOROWSKI & FISHBACK from normal cases.

The conditions affecting the osmotic resistance in the erythrocytes in cardiac insufficiency are, no doubt, multifarious. From the present observations, it is evident that both the minimal and the maximal resistance increases with the *MCD* in the groups of mild insufficiency. As far as the maximal resistance is concerned, this conforms to Larsen's forementioned investigations of hepatitis. Thus, an occurrence of blood corpuscles of a diameter of more than normal implies an elevation of the maximal resistance. According to this it must be mentioned that the present investigation has shown that simultaneously an increased amount of erythrocytes is to be found. Thus it seems probable that at regenerative blood formation in congestive heart failure bigger erythrocytes with increased resistance are introduced into the blood stream.

This aspect agrees very well with those of KORWITZ & PRATT; HAMI & PRATT; SATTLER; SIMMEL; SNAPPER; ODA; HANDOWSKY; WILBRANDT & HERRMAN; SCHÖNHOLTZER & LÜTHI; VANNOTTI & MARKWALDER, obtained from other hematologic fields.

On the other hand, as pointed out by MELDOLESI, BUCCIANI and ROSSI, WALLER et al. and TSAI CHIAO, CHEN & CHIU peripheral factors are not unlikely to exert an

influence. In the present investigations, no correlation has proved noticeable between the *MCD* and the resistance in cases with the most pronounced decompensation. This may indicate an influence from other factors of, e.g., a peripheral nature, such as osmotic changes. According to WALLER and SIMMEL it seems unlikely that the changes in the CO_2 and O_2 tensions in cardiac congestion could produce any variations in resistance in vivo.

The reduced minimal resistance is, possibly, to be explained from the fact that old and small cells are hemolyzed more rapidly than normal ones, probably owing to peripheral factors and the increased hemocatabolic capacity of the spleen during a stasis (BJÖRKMAN 1947), a suggestion propounded also by FRANCESCON.

In the course of a treatment, the resistance changes, the maximal resistance abating, and approximating to normal values, once compensation has been attained, while the minimal resistance remains slightly reduced.

It is, for this reason, probable that some normal-sized cells are all the time subject to various influences, involving a reduction in the minimal resistance and making the erythrocytes hemolyze more rapidly. Also in cases where a so-called clinical compensation has been reached, peripheral factors and the spleen are likely to play a part in the reduction of the osmotic resistance of the erythrocytes.

ON SERUM IRON

With due regard paid to the dilution effect from the increased plasma volume, the valvular heart affection will be found in decompensated cases to display, on an average, rather normal values for serum iron, while the vascular cases show subnormal values. Just as in normal conditions, this material reveals lower values for serum iron for women than for men.

It is difficult to explain the difference that apparently occurs between vascular and valvular heart affections. For such a purpose, a bigger material would be wanted. The mean age in the vascular group is higher than that of the valvular cases. But within the observed range of 50—77 years, no correlation can be traced in the vascular group between the values for the iron and the age. However, DAHLBERG & JOSEPHSON were able to show that in normal men and women between 70—80 years of age the serum iron values were significantly lower than in persons under 50. Whether or not the enteral absorption conditions or frequency of achylia differ between the two groups of heart affections is a point that cannot be decided. To judge from LINTZEL & RADEFF's, as well as GRANICK's, investigations, the anoxia in the intestinal mucosa is not a determining factor, though the question of the part possibly played in the absorption conditions by an edema in an intestinal wall has to be left open. Still, supposing this to be an actual fact, the values for the serum iron would, to judge from the investigations of HUFF and others, probably be affected but inconsiderably, since in all likelihood less than 1 per cent of the total turnover of plasma iron is normally concerned with absorption.

It is, on the other hand, worth noting that, in spite of the presence of a hepatic injury in a cardiac insufficiency, no hypersideremia is to be found, such as, according to some authors, is noted in acute cases of hepatitis and sometimes in a liver cirrhosis. It might therefore, be assumed that, in cardiac insufficiency, the iron depots have not lost their capacity to store iron, an interpretation occasionally adduced in cases of hepatitis. According to certain investigations on autopsy material (LUBARSCH, NORDMEYER) as well as to animal experiments in low-pressure chambers (LINTZEL & RADEFF) and at high altitudes (VANNOTTI), these depots would contain rather less iron, in cardiac insufficiency and conditions of anoxia, than in normal cases. In the opinion of NORDMEYER, LINTZEL & RADEFF and VANNOTTI, the explanation is to be found in an increased demand for iron in the polycythemic reaction in these conditions. LINTZEL & RADEFF also noted increases in the hemoglobin iron. VANNOTTI observed increased iron in the bone marrow. In the view of LINTZEL & RADEFF, the total of iron is unchanged. Only a deviation in the deposited amount has, they contend, taken place. From their investigations they conclude that no increased excretion of iron has taken place.

Such a deviation can, probably, be reflected in the values for the serum iron. During blood regeneration at high altitudes VANNOTTI & MARKWALDER found that the serum iron decreased. On a later occasion VANNOTTI found the circulating iron slightly higher in altitude hypoxemia than in normal animals.

From the here recorded investigations it also appeared that a significant correlation occurred in the total corpuscular volume and serum iron in one of the groups of cardiac insufficiency. The same tendency, though not statistically significant, was to be noted in the other groups. The probability of this result from a medical point of view is not contradicted by NORDMEYER, LINTZEL & RADEFF's, VANNOTTI & MARKWALDER's and VANNOTTI's observations. They further agree with the investigations by HUFF and others of secondary polycythemias in which an increased red cell iron turnover was ascertained. According to HUFF and others, this could reasonably be explained by an increase in the amount of blood corpuscles and, possibly, a shorter length of life in these erythrocytes or some disturbance in the hemoglobin synthesis.

It should, further, be noted that from the present investigations, as well as from those of other authors, it has emerged that, in cases of cardiac decompensation, a diminished resistance is to be found in certain blood corpuscles. This should have affected the recorded significant correlation between serum iron and bilirubin. Still, an increase in bilirubin does not in itself constitute any proof of an increased hemolysis. When the liver is intact, VAHLQVIST (1941), to take an instance, failed to observe any increase in bilirubin in induced hemolysis by phenylhydrazine, but in cardiac insufficiency there is no doubt about the presence of a hepatic injury.

On the occasion of a compensation, increased values for serum iron are to be noted. As seen from the results of the investigations, the increased values cannot only be attributed to any increased concentration. At a compensation, the osmotic resistance of the blood corpuscles likewise decreases. This decrease is, at any rate, statistically significant as far as the maximal resistance is concerned. A significant correlation has been ascertained

between the latter and serum iron at compensation. From this, the increased values for serum iron can be supposed to originate, *inter alia*, in a growing hemolysis of blood corpuscles. As seen from the present investigation, also the total red cell volume is, at compensation, found to be reduced.

Yet, increased serum iron values at compensation may also be explained from the fact that, at a decreased production of erythrocytes, a surplus of iron, reflected in elevated values for serum iron, is to be found. This mechanism is probably also to be noted when, during a compensation the reticulocyte values are normal and the erythropoietic activity in the bone marrow, as shown by ORT, is lower than during a decompensation.

These two explanations of the increase in the amount of serum iron at compensation are, probably, only to be regarded as two ways of looking at the same fundamental cause, seeing that, at achieved compensation, a decrease takes place in the *MCD* of the red blood corpuscles, as well as in the maximal resistance, as seen from the investigations. In not too grave cases of cardiac insufficiency, the *MCD* will also be seen to be correlated to the osmotic resistance. Further, from the point of view of time, a diminution of total red cell volume will fairly well coincide with a decrease of the maximal resistance and of the *MCD* of the erythrocytes. It, accordingly, seems likely that an increased erythropoietic activity originates erythrocytes with a bigger *MCD*, higher maximal resistance and a shorter term of life than will be found normally. Once the production of such cells has fallen, while an increased hemolysis of other erythrocytes continues, an increased content of serum iron will appear.

REMARKS ON THE HEMOGLOBIN SYNTHESIS

Studies regarding the hemoglobin synthesis form a subject separate from that of erythrocyte regeneration. It cannot be closely elucidated in the present investigations. A few remarks may, however, be made.

As previously stated, no correlation has been traceable between the *Hb* and the total red cell volume, partly on account of a simultaneous dilution effect through an increase in the plasma volume.

Apart from this dilution factor, the *Hb* value is conditioned by the amount of erythrocytes and the amount of hemoglobin of the individual blood corpuscles.

The total volume of blood corpuscles has been shown to increase during cardiac insufficiency.

During decompensation, about normal values for the *MCH* will be found and significant lower values for the *MCHC*. Moreover, the *MCHC* values are inversely correlated to the mean corpuscular volume while no correlation is noticeable between the *MCH* and the *MCV*.

In an earlier chapter it has been already pointed out that there is reason to presume that the lowered value of the *MCHC* indicates that the corpuscular maturation is incomplete in cases of cardiac insufficiency, while on the other hand, the hemoglobin synthesis is hardly, to any more notable degree, disturbed, seeing that, broadly speaking, the *MCH* displays normal values.

The supply of iron is important for the hemoglobin synthesis. In the vascular groups of cardiac insufficiency, serum iron values below normal have been noted, even though regard has been paid to the effect of dilution, while, in the valvular groups, normal values have, on the whole, been recorded. Still, this difference in the values of the serum iron does not seem to have exerted any particularly striking influence on the hemoglobin values, seeing that no difference in the *MCHC* and *MCH* has been observed between vascular and valvular men. From this, the values of the serum iron would hardly seem to reflect any deficiency in the hemoglobin synthesis in a material of cardiac insufficiencies, although, in individual cases with conspicuously low values of serum iron, such a possibility cannot be excluded. WILTON and others have pointed out that low *MCHC* values in connection with a corpuscular regeneration do not indicate an anemia. Low values of *MCHC* have also been noted at the increased erythropoiesis that occurs at high altitudes.

It is beyond the scope of this investigation to enter into the question whether any finer changes occur in the hemoglobin synthesis in cardiac insufficiencies.

It should be noted that an increased amount of free erythrocyte protoporphyrin has been observed during decompensation, though in a small material. An increase has been supposed to suggest a deficient hemoglobin synthesis, originating in, e.g., a deficiency of iron (SEGGER, CARTWRIGHT, HUGULEY, ASCHENBRUCHER, FAY & WINTROBE, 1948) or an increase of immature erythrocytes (GRINSTEIN, SILVA & WINTROBE, 1948, STASNEY & MC CORD 1943, and others). At any rate in the present material of cardiac insufficiencies, the presence of immature erythrocytes and increases in the number of reticulocytes in the peripheral blood have been observed. From the present investigations, it cannot be decided whether also other factors are active.

It should also be kept in mind that, in cardiac insufficiency, a deviation in the dissociation curve of the oxyhemoglobin is to be noted.

Similar observations have been made in cyanotic congenital heart disease (MORSE, CASSELS & HOLDER, 1950) as well as at high altitudes (KEYS, HULL & GUZMAN BARRON 1936).

Summary

1°) In the Introduction, the purpose and intention of the present investigations have been defined. The principal aim has been to shed light on the erythropoiesis in cardiac insufficiency and on its results as they appear in the peripheral blood, in reticulocyte numbers, total red cell volume and the size of individual blood corpuscles. In addition, the osmotic resistance of the blood corpuscles and the serum iron have been subjected to study, and certain data regarding the hemoglobin synthesis have also been included. Comparisons have been made between the P^{32} and the Evans blue methods with regard, *inter alia*, to the total red cell volume.

2°) The material of cardiac insufficiency studied in the present investigation comprises cases, classified according to sex and diagnosis. Two diagnostic groups have been formed, one embodying cardioarteriosclerotic and hypertensive heart diseases, the other, valvular heart diseases, i.e. mitral or combined mitral-aortic valvular defects. The age distribution of the material has also been set forth. In each group of cardiac insufficiency, the material has been divided according to sex and the degree of decompensation.

3°) Comparative values for the total red cell volume have been obtained from a control material comprising 35 men and 7 women, significantly higher values being noted for men. The results are in accordance with those from investigations recorded in the literature, in which labelling methods with radioactive phosphorus or iron have been applied.

4°) Investigations regarding the P^{32} and Evans blue methods are reported. Errors of the method are discussed. The error of the procedure here adopted (inherent in a stated value for the total red cell volume), according to the P^{32} method, has been estimated at 2.3 per cent.

5°) From observations of the mixing conditions, it seems likely that, as far as the blood corpuscles are concerned, the mixing in the major vascular areas is completed within 10–12 minutes in decompensated cases. Further, from results of the author's own work as well as from data in the literature, it seems probable that all blood corpuscles are in active circulation. In decompensated cases, the cell volume recorded by the P^{32} method has been found to be about 18 per cent lower than that resulting from the Evans blue-venous hematocrit method. The ratio between body and venous hematocrit, has been estimated at 0.90. The mixing conditions for the Evans blue are discussed.

6°) In decompensation, the bone marrow shows a comparatively greater percentage of nucleated red blood corpuscles than normal. Apparently, this increase corresponds,

numerically, to a recorded increase of the orthochromatic erythroblasts. With regard to the percentage number of erythroid cells and the percentage distribution of the various erythroblastic cells, the values observed in vascular and valvular heart affections seem to correspond. Nevertheless as far as the vascular affections are concerned, certain differences appear to occur in the distribution of the mitotic numbers with a tendency to higher mitotic numbers in young erythroblastic cells. Within all the erythroblastic cells, macroforms are noted, disclosing a percentage distribution that differs between vascular and valvular heart affections. A correlation has been ascertained between the number of nucleated red blood corpuscles and the arterial oxygen saturation. Arising from this and a survey of the literature available in this field of research, the part played by anoxemia in the regulation of the erythropoiesis is closely examined. Very possibly, it is the anoxia, together with some endocrine factor, that elicits the polycythemic reaction.

7°) A correlation has also been traceable between arterial oxygen saturation and the reticulocytosis that occurs in cardiac insufficiency.

8°) In decompensation, both men and women in the two groups of heart affections reveal total red cell volume significantly higher than the normal. Numerical, though not significant, differences are found between the sexes and between the two groups of heart affections, males and cases of valvular heart affections, showing higher total red cell volume. A study of the factors conditioning this increase in the total red cell volume suggests that the cell volume increases with the duration of the insufficiency symptoms of the valvular heart affections, while on the other hand the vascular cases disclose comparatively high values for the total red cell volume even after a short duration of manifest symptoms of insufficiency. In the latter group, a tendency to increase in the total red cell volume commensurate with the degree of decompensation is noticeable, cases of the most benign degree of decompensation showing no significant difference from the values in the control group.

The different conditions which may affect the increases in the total red cell volume in valvular and vascular heart diseases, respectively, have been compared with the findings in the bone marrow and are fully discussed in the light of the clinical divergences in the development of a cardiac insufficiency in those two types of heart affections.

An iron deficit, suggested as a limit for red cell regeneration, is also discussed.

9°) In decompensation an increased plasma volume is observed which decreases at compensation. As in normal cases a higher plasma volume is obtained in decompensated men than in women.

10°) In cardiac insufficiency, a macro-normoblastic erythropoiesis is noted. In the peripheral blood, anisocytosis is observed. In addition, the *MCD* of the red blood corpuscles displays elevated values as against a normal material.

The pathogenesis of the macrocytosis is discussed on the basis of the author's own clinical investigations, animal experiments (PLUM and HEDLUND) and other researches reported in the literature. The macrocytes seem to have a central hematopoietic genesis. The possibility of certain influences, in the form of peripherally active factors, is not ruled

out. In this connection, the finding of an increasing content of fluid in the erythrocytes commensurate with the degree of insufficiency is discussed. It is, however, at the same time pointed out that an increase in the fluid content may occur at a regeneration of blood corpuscles with the appearance of macrocytes. Apparently, a not inconsiderable part in the pathogenesis is played by the duration of the insufficiency symptoms and the degree of the decompensation. The effect of the function of the liver on the *MCD* is discussed. Nevertheless no correlation between certain liver tests and the *MCD* has been detectable. The venous stasis and, not improbably, some endocrine factors constitute, in all likelihood, the basis of the pathogenesis. Proceeding from the hypothesis that the erythrocytes originate from more or less mature erythroblasts, interpretations are propounded of the findings of polychromasia and reduced values for the *MCHC* in the erythrocytes, as well as of the practically normal *MCH* values. Normally, the development from polychromatic to orthochromatic erythroblasts brings about an increase in the *MCHC*, by means of a volume decrease, while, broadly speaking, the *MCH* remains unchanged. The forementioned hypothesis is further discussed in connection with the recorded findings of a correlation between the orthochromatic/polychromatic erythroblasts and the *MCD* of the erythrocytes, together with the absence of any ascertainable correlation between the forementioned quotient and the corresponding *MCH* values.

The observed difference in the values of the serum iron between vascular and valvular male cases does not seem to have affected the hemoglobin synthesis in these groups to any noteworthy degree, considering that, on an average, conforming values are to be noted for the *MCHC* and *MCH*, respectively.

11°) When examining certain hematologic findings, observed at decompensation, an intensified erythropoiesis is noticed, resulting in an elevated total red cell volume and a reticulocytosis. In the peripheral blood macrocytes appear, characterized by an elevated osmotic resistance, while, simultaneously, other erythrocytes with a reduced resistance are noted. The origination of the reduced minimal resistance is fully discussed. In this connection, particular attention has been paid to the influence of peripheral factors and the increased hemocatatonic effect of the splenic stasis. The degree of hemolysis that is to be found manifests itself, inter alia, in a correlation between bilirubin and serum iron. For some groups of insufficiency, at any rate, the iron values keep conspicuously low. The question of the significance of this is discussed in detail, the regeneration of blood corpuscles being supposed to carry with it an increased demand for iron for the hemoglobin synthesis. It is, however, pointed out that, with regard to recorded differences between the groups of vascular and valvular heart affections, an age factor may assert itself.

The changes in the hematologic process, during a treatment that leads to compensation, have been studied. The total red cell volume, the *MCD* and the maximal resistance are found to fall in the direction of normal values while at the same time, the serum values increase. This has been proved not to originate solely in a decreased plasma volume. The erythropoietic activity is diminished, a fact reflected in the reticulocyte

curve. These results are discussed. Probably, a population of macrocytes, with a supposed shorter lease of life than normal, disappears from the circulation. It is in this light, that the increase in serum iron has to be viewed. In cases, on the other hand, in which an evident clinical improvement in the condition of decompensation can hardly be said to have occurred with the treatment, the above discussed conditions remained, broadly speaking, unchanged during the period of observation.

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Owing to the comprehensiveness of the literature dealing with the present problems, only some of the numerous research-workers have to be quoted in the text. However, the references given in this book are too numerous to admit of a complete list. Such authors as are quoted, but not included in the list, will be found referred to in the chief works on the same subject.

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